Nutritional supplementation with dietary omega-3 PUFA-rich flaxseed and canola oils enhances prime lamb performance and meat quality

Thesis submitted by

Don Viet Nguyen

BAnVetSci (Vietnam National University of Agric)

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College of Public Health, Medical and Veterinary Sciences

James Cook University

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Supervision

Assoc. Prof. Aduli Malau-Aduli

Prof. Peter Nichols

Assoc. Prof. Bunmi Malau-Aduli

Assoc. Prof. John Cavalieri

Statistical and Analytical Support

Assoc. Prof. Aduli Malau-Aduli

Prof. Peter Nichols

Dr. John Otto

Dr. Aaron Flakemore

Editorial Support

Assoc. Prof. Aduli Malau-Aduli

Prof. Peter Nichols

Assoc. Prof. Bunmi Malau-Aduli

Assoc. Prof. John Cavalieri

Declaration on Ethics

The research presented and reported in this thesis was conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th edition, 2013 and the Tasmania Animal Welfare Act, 1993. The research received animal ethics approval from the University of Tasmania Animal Ethics Committee (Permit Number A13839).

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Abstract

This thesis tested the effects of supplementation with graded levels of either canola or flaxseed oils and their interactions with lamb breed and sex on Australian prime lamb productivity and product quality. It was hypothesised that supplementation of prime lambs with oil would interact with lamb breed and sex to: (1) positively impact feed intake, body conformation, growth and carcass indices of lamb productivity; and (2) alter plasma metabolites, wool and meat eating quality traits, and the fatty acid (FA) profiles of lamb tissues and organs including *Longissimus dorsi* muscle, visceral adipose tissue, heart, liver and kidney. Independent effects of breed and sex on productivity and product quality of lambs were also investigated.

Sixty dual-purpose prime lambs comprising purebred Merinos and Corriedale x Merino and White Suffolk x Merino crossbreds were allocated to one of five treatments of lucerne hay basal diet supplemented with isocaloric and isonitrogenous wheat-based pellets. Treatments were: no oil inclusion (Control); 2.5% canola oil; 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil, with lamb groups balanced by breed and gender. Each lamb was daily supplemented with one kg of pellets and had free access to lucerne hay and water throughout the 7-week feeding trial, after a 3-week adaptation. Individual animal basal and supplementary pellet feed intakes were recorded daily, while body conformation traits, body condition scores and liveweights were measured on days 0, 21, 35 and 49. The lambs were dye-banded on the mid-side and shorn before commencing the feeding trial and mid-side wool samples were collected from the same dye-banded area of each lamb at the end of the experiment. Plasma metabolites, fatty acids and carcass data were determined from blood, muscle, adipose, heart, kidney and liver samples at slaughter. All investigated parameters were analysed using the Statistical Analysis System (SAS) software version 9.2. General Linear Model (PROC GLM) analyses were used to fit oil supplementation, lamb breed, gender and their second-order

interactions as fixed effects and measured traits associated with growth, body conformation, carcass characteristics wool, plasma metabolites and FA profile as dependent variables. In each case, linear and cubic orthogonal contrasts were interrogated and where any fixed effect was not a significant factor, it was removed from the final analytical model since it was not a variance contributor to the observed variation.

It was evident from the series of 5 experimental studies that supplementation of prime lambs with graded levels of canola and flaxseed oils, breed and sex significantly influenced productivity and product quality in Australian prime lambs, in which:

(1) Oil supplementation had no detrimental effect on growth performance, body conformation, wool quality, carcass characteristics, plasma metabolites and sensory attributes of meat eating quality;

(2) the supplementation of 5% flaxseed oil or canola oil into lamb diets decreased the omega-6/omega-3 (n-6/n-3) ratio and increased the content of the health beneficial omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA) in *Longissimus* muscle;

(3) Supplementing of 5% flaxseed oil into lamb diets reduced n-6/n-3 ratio in all investigated tissues and improved total n-3 PUFA composition in kidney, liver and visceral adipose;

(4) meat from lamb fed 5% flaxseed oil or canola oil reached the claimable 'source' level of n-3 LC-PUFA, and liver and kidney could be consumed as alternative 'good sources' of n-3 LC-PUFA;

(5) lamb breed and sex were imperative to influencing lamb growth, carcass, wool and meet eating quality, FA profiles of edible tissues and plasma metabolites; and

(6) interaction effects between oil supplementation and lamb breed on ADG and feed conversion ratio, visceral adipose and heart FA composition and meat juiciness score were detected. As a result, the hypothesis was accepted.

In conclusion, both canola and flaxseed oils can be effectively used in the prime lamb industry without any detrimental effect on productivity and product quality during a ten-week feedlot period. Furthermore, the observed interaction effects of oil supplementation with breed permit flexibility in operational options of optimising profitability from meat in prime lamb production. These findings suggest that prime lamb producers can better manage and match their breeding goals with feed resources. Therefore, a combination of 5% oil supplementation and lamb genetics is an effective and strategic management tool for improving feed efficiency, growth performance, and the content of n-3 LC-PUFA in lamb meat.

List of Publications from Thesis

Peer-reviewed Journal Papers

1. Nguyen, D. V., Le, V. H., Nguyen, Q. V., Malau-Aduli, B. S., Nichols, P. D., Malau-Aduli, A. E. O. (2017a). Omega–3 Long-chain fatty acids in the heart, kidney, liver and plasma metabolite profiles of Australian prime lambs supplemented with pelleted canola and flaxseed oils. *Nutrients*, Vol 9, Issue 8, p893-899. doi:10.3390/nu9080893

2. Nguyen, D. V., Malau-Aduli, B. S., Nichols, P. D., Malau-Aduli, A. E. O. (2018a). Growth performance and carcass characteristics of Australian prime lambs supplemented with pellets containing canola oil or flaxseed oil. *Animal Production Science.* doi: https://doi.org/10.1071/AN16812

3. Nguyen, D. V., Le, V. H., Nguyen, Q. V., Malau-Aduli, B. S., Nichols, P. D., Malau-Aduli, A. E. O. (2018b). Feed intake, growth and wool quality responses of genetically divergent Australian lambs to canola oil or flaxseed oil supplementation, *PLoS ONE* (in press).

4. Nguyen, D. V., Malau-Aduli, B. S., Nichols, P. D., Malau-Aduli, A. E. O. (2018c). Supplementation with plant-derived rich in omega-3 polyunsaturated fatty acid sources for lamb production: A review, *Veterinary and Animal Science* Volume 6, (December Issue), pages 29-40. doi: https://doi.org/10.1016/j.vas.2018.08.001

5. Nguyen, D. V., Flakemore, A. R., Otto, J. R., Ives, S. W., Smith, R. W., Nichols, P. D., Malau-Aduli, A. E. O. (2017b). Nutritional value and sensory characteristics of meat eating quality of Australian prime lambs supplemented with pelleted canola and flaxseed oils: Fatty acid profiles of muscle and adipose tissues. *Internal Medicine Review*, Volume 3, Issue 3, pages 1-21. doi: http://dx.doi.org/10.18103/imr.v3i3.295

Conference Paper

6. Nguyen, V. D., Ives, S. W., Smith, R. W., and Malau-Aduli, A. E. O. (2015). Growth response of purebred Merino and crossbred prime lambs supplemented with canola and flaxseed oils. In: *Proceedings of the 5th International Conference on Sustainable Animal Agriculture for Developing Countries*, pp. 113-116, 27-30 October 2015, Pattaya, Thailand.

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List of Abbreviations

ABARES = Australian Bureau of Agricultural and Resource Economics and Sciences

ABS = Australian Bureau of Statistics

ACIAR = the Australian Centre for International Agricultural Research

ADF = acid detergent fibre

ADG = average daily gain

AG = Australian Government

ALA = alpha-linolenic acid

ASBVs = Australian Sheep Breeding Values

AV = Agriculture Victoria

AWI = Australian Wool Innovation

BCS = body condition scores

BCTRC = boneless, closely trimmed retail cuts

BH = biohydrogenation

BHB = beta–hydroxybutyrate

BL = body length

BLUP = Best Linear Unbiased Prediction

BWT = body wall thickness

CCW = cold carcass weights

CF = comfort factor

CFY = clean fleece yield

CG = chest girth

CHD = Coronary heart disease

CL = chilling loss

CP = crude protein

CSIRO = Commonwealth Scientific and Industrial Research Organization

CV = coefficient of variation

CVD = cardiovascular diseases

DHA = docosahexaenoic acid

DM = dry matter

DMI = dry matter intake

DP = dressing percentages

DPA = docosapentaenoic acid

EBV = Estimated Breeding Values

EE = ether extract

EPA = eicosapentaenoic acid

FA = fatty acids

FAME = fatty acid methyl esters

FAO = Food and Agriculture Organization

FC = fibre curvature

FD = fibre diameter

FDe = fat depth

FE = feed efficiency

FSANZ = Food Standards Australia New Zealand

FSD = fibre standard deviation

FSSA = French Food Safety Agency

GC = gas chromatograph

HCW = hot carcass weight

IMF = intramuscular fat

JCU = James Cook University

JMHLW = Japanese Ministry of Health, Labour and Welfare

LTL = *Longissimus thoracis et lumborum* muscle

LWT = liveweight

MLA = Meat and Livestock Australia

MUFA = monounsaturated fatty acids

n-3 LC-PUFA = omega-3 long-chain polyunsaturated fatty acids

n-3 PUFA = omega-3 polyunsaturated fatty acids

n-6 PUFA = omega-6 polyunsaturated fatty acids

ND = not detected

NDF = neutral detergent fibre

NFC = Non-fibrous carbohydrates

NHFA = National Heart Foundation Australia

NHMRC = National Health and Medical Research Council

OM = organic matter

PUFA = polyunsaturated fatty acids

REA = ribeye area

SAS = Statistical Analysis System

SF = spinning fineness

SFA = saturated fatty acids

SGA = Sheep Genetics Australia

UFA = unsaturated fatty acids

UTAS = the University of Tasmania

WH = wither height

WHO = World Health Organization

Chapter 1: General Introduction

Australia remains the world's largest producer and exporter of wool with the world's best quality woollen fibre (Abbott, 2013; Leith, 2014). Australia is also the largest exporter of sheep meat and the second largest sheep producer in the world (MLA, 2017b). Governor Philip brought the first flock of 29 sheep into Botany Bay, Australia from the Cape of Good Hope, South Africa in 1788. The national sheep population is currently 67.5 million head (ABS, 2017a) accounting for around 25% of world wool production and approximately 8% of the world's lamb and mutton supply. The economic value of the Australian sheep industry was $6.2 billion in 2016 (ABS, 2017b), contributing approximately 11% of the total $56 billion farm value in 2016 (ABS, 2017a).

In the past two decades, dual-purpose sheep production systems with both wool and meat production goals have been adopted by the Australian sheep industry (Rowe, 2010; Malau-Aduli and Akuoch, 2012). This dual-purpose system enables producers to deal with individual market volatilities (Kopke et al., 2008; Malau-Aduli et al., 2012a), and increase production and genetic cost efficiencies (Ingham et al., 2007). Sheep Genetics Australia (SGA) has mainly employed LAMBPLAN and MERINOSELECT to advise breeders in order to drive genetic advantages in nucleus flocks. Such advantages then flow through to commercial flocks via producers (Walkom and Brown, 2017). The Australian sheep industry has increasingly used the complexity of breeding objectives to generate sheep with desired growth, meat, reproductive prolificacy and wool quality traits (Brown and Swan, 2016).

Lamb production in Australia is generally based on extensive grazing systems that can include dryland, senesced, green and irrigated pastures (Ponnampalam et al., 2014b). The

extensive production systems are diverse and are determined mainly by environmental conditions (rainfall, climate and soil types) and survival of the pasture species, which all influence feed availability and pasture growth. Animals raised in these systems tend to have slower growth rates, thus affecting production efficiency and the ability to reach target slaughter and carcass weights within a particular period (De Brito et al., 2017). It is often necessary to provide supplements to satisfy the nutritional requirements of sheep for optimal growth and carcass production (Turner et al., 2014). Generally, lambs fed concentrate-based diets have greater growth rate than those grazing pasture-based diets only (Bessa et al., 2008; Armero and Falagán, 2015; De Brito et al., 2017). It has been shown that although genetics and sex affect meat characteristics (Gruffat et al., 2013; Malau-Aduli et al., 2016), the composition of diet is also a major factor influencing nutritional value and sensory properties of meat (Asadollahi et al., 2017; Chikwanha et al., 2017). Thus, intensive supplementation of prime lambs with concentrate during the finishing period has become a common practice to improve both animal and paddock productivities and to meet a wider range of market demands (Watkins et al., 2013; Frank et al., 2017). The selection of supplementary feeds in sheep production should not only be cost-effective, but also be sustainable, minimising competition for food with humans and impacts on the environment, animal health and welfare.

Sheep meat is an excellent nutritional source of essential amino acids and provides a wide range of important micronutrients including iron, zinc and B vitamins (Wyness, 2016). However, Enser et al. (1996) reported that lamb had a greater fat percentage in comparison with other protein sources and lower levels of polyunsaturated fatty acid (PUFA) in comparison with seafood or pork. The negative effects, perceived by some, although not all, on human health overshadow the benefits associated with consumption of fat derived

from lamb (Chikwanha et al., 2017). High consumption of red meat has been proposed to result in increased incidences of central nervous system disorders, cardiovascular diseases and cancers (Ekmekcioglu et al., 2017; Wolk, 2017). This perception emanates from its high levels of saturated fatty acids (SFA) and low content of omega-3 polyunsaturated fatty acids (n-3 PUFA) (Bessa et al., 2015).

It is evident that omega-3 long-chain (≥ C_{20}) polyunsaturated fatty acids (n-3 LC-PUFA) are intrinsic components of all cell membranes in the body (Gorjão et al., 2009; Khan and He, 2017). They play critical roles in the constitution and development of brain and retinal tissues (Swanson et al., 2012; Walker et al., 2015), and in the prevention of and reduction of the risk of chronic diseases in humans (Nichols et al., 2014; Walker et al., 2015; Watanabe and Tatsuno, 2017). Various recommendations for daily n-3 LC-PUFA intake have been proposed and consumers have also become more aware of the health benefits of these fatty acids (FA). However, Western diets have been reported to be severely lacking in these FA (Fayet-Moore et al., 2015; Salem and Eggersdorfer, 2015). Although a major source of n-3 LC-PUFA is seafood, it is not a regular part of the traditional diet in many Western countries (Byelashov et al., 2015). Australians consume approximately 130 g of meat daily (including 57 g of red meat), but only 22 g of seafood (Sui et al., 2016). Thus, the ability to increase n-3 LC-PUFA content in other human foods such as red meat, is another pathway available to meet the recommended intakes of these FA.

The use of innovative nutritional strategies to improve PUFA content in ruminant tissues has been reported (Bessa et al., 2015; Ponnampalam et al., 2015; Jaworska et al., 2016; Asadollahi et al., 2017). These reports demonstrated that n-3 PUFA content in meat products can be manipulated by supplementing ruminants with feeds enriched with n-3 PUFA dietary sources. The richest sources of n-3 PUFA supplied in ruminant diets include

fish, algae, oilseeds and their oils (Woods and Fearon, 2009; Nichols et al., 2010). The practical inclusion of marine products in ruminant supplements is problematic because of concerns regarding detrimental effects on sensory eating quality (Scollan et al., 2014; Urrutia et al., 2016), prohibitive costs and resource scarcity (Lenihan-Geels et al., 2013; Kitessa et al., 2014). Thus, oilseeds and their oils are considered as alternative and sustainable sources of dietary n-3 PUFA. Canola and flaxseed oils contain an abundance of alpha-linolenic acid (ALA, 18:3n-3) (Gillingham et al., 2011; Ding et al., 2017) and have been of interest in feeding trials aiming to increase n-3 PUFA levels in lamb (Urrutia et al., 2015; Francisco et al., 2016; Flakemore et al., 2017).

The main objective of inclusion of lipid in ruminant diets is to increase the content of health-benefitting PUFA in meat for human consumption. However, the consumption of non-carcass components such as heart, liver and kidney, is very common in many countries. Furthermore, offals can be cheap sources of proteins, minerals and vitamins (Toldrá et al., 2012; Umaraw et al., 2015) and play an important role in processed product formulations (Umaraw et al., 2015). For instance, liver and kidney are typically used to produce pies and other processed food products. Thus, lamb edible products include not only meat, but also these visceral organs. Studies investigating FA profiles of lamb heart, liver and kidney tissues remain scarce apart from the reports of Kashani et al. (2015) and Malau-Aduli et al. (2016). It is also necessary to take into account the feed intake, growth and health responses of animals to n-3 PUFA supplements, as well as their meat eating quality, because changes in dietary fat can produce differences in animal health, productive performance and sensory properties (Chikwanha et al., 2017). Bessa et al. (2015) stated that maximising the content of n-3 LC-PUFA in ruminant products would be a highly desirable production target to

enhance nutritional quality. However, the biohydrogenation of these FA that occurs in the rumen presents challenges that need to be addressed.

Ideally, an increase in n-3 LC-PUFA content should be achieved simultaneously without detrimental impacts on the productive performance of animals and the sensory attributes of meat products. Although, numerous studies have been conducted in this area, the relationships between the enrichment of n-3 PUFA in lamb diet, and their health, productive performance, as well as sensory quality are not yet fully understood.

Genetic management through crossbreeding strategies, provides a cumulative and long-term alternative approach to nutritional manipulation (Malau-Aduli et al., 2014). The effects of genetics on growth responses, meat nutritional values, eating quality and wool traits have been investigated (Holman et al., 2014a; Holman et al., 2014b; Gardner et al., 2015; Walkom and Brown, 2017). However, these studies were conducted under grazing conditions with seasonal variability occurring in pasture supply. Research simultaneously studying the effects of both nutrition and genetics on lamb performance and their products (edible tissues and wool) in an on-farm intensive management system during finishing periods remains limited. Additionally, it is evident from published literature that there are existing knowledge gaps in the integrated approach of the use of appropriate supplementary levels and the associated impacts of flaxseed and canola oils on Australian dual-purpose prime lambs from different genetic backgrounds. Therefore, the series of studies reported in this thesis are based on the use of an on-farm intensive management system, with the overarching objectives of this research project being to investigate:

1) Feed intake, growth, carcass and wool quality responses of Australian dual-purpose prime lambs to supplementation with canola oil or flaxseed oil;

2) Variations in plasma metabolites, meat eating quality and FA profiles in the muscle, adipose, heart, kidney and liver tissues; and

3) Second-order interactions between oil supplementation, breed and gender combinations in prime lambs.

These objectives were established in an attempt to provide sheep farmers with a variety of choices for targeting optimal slaughter and carcass weights, meat nutritional values, eating quality and wool traits under the same intensive finishing management system. Therefore, this thesis is structured into the following chapters:

Chapter 1: General Introduction

Chapter 2: Literature Review: An in-depth and systematic exploration of published literature on current background of the Australian sheep industry, genetic management, contribution of n-3 LC-PUFA consumption to human health, effects of ALA-rich dietary supplementation on lamb health and productivity, quality of edible tissues including n-3 LC-PUFA content and meat sensory quality attributes.

The successive chapters are investigative experimental studies that describe the effects of dietary supplementation with graded levels of either canola or flaxseed oils on feed intake, growth, carcass and wool quality, plasma metabolites, meat eating quality and FA profiles in a range of body tissues in both ewes and wethers derived from Australian dual-purpose prime lambs.

Chapter 3: The main objective of this chapter was to investigate the effects of supplementation with graded levels of either canola oil or flaxseed oil, lamb breed, gender and their second-order interactions on feed intake, variations in growth and wool quality. It also aimed to estimate residual phenotypic correlations within and between wool quality

traits and lamb performance under the same management system. The hypotheses tested were: (1) canola or flaxseed oil supplementation will not have detrimental effects on feed intake, variations in growth performance and wool quality of lamb, (2) lamb breed will affect both growth performance and wool quality, (3) the pre-slaughter attributes and wool quality will be influenced by gender, and (4) there will be some second-order interaction effects on feed intake variations in growth performance and wool quality.

Chapter 4: The main objective of this chapter was to investigate the effects of supplementation with graded levels of either canola oil or flaxseed oil, lamb breed and their second-order interactions on feed efficiency, growth performance, body conformation and carcass characteristics. This chapter tested the hypothesis that feed efficiency, growth performance, body conformation and carcass characteristics of Australian dual-purpose prime lambs will be influenced by different breeds, elevated supplemented levels of either canola oil or flaxseed oil pellets in an intensive finishing management system, and that there would be significant phenotypic correlations between body conformation and growth traits under the same management system.

Chapter 5: The major objective of this chapter was to investigate the FA profiles of *Longissimus thoracis et lumborum* (LTL) muscle and visceral adipose tissues, and sensory traits of meat from Australian dual-purpose prime lambs supplemented with graded levels of either canola oil or flaxseed oil enriched pellets. The following hypotheses were tested: (1) Pellets enriched with canola or flaxseed oils will enhance n-3 LC-PUFA profiles to meet the values considered more optimal for human diets and will not adversely influence sensory properties of lamb, and (2) lamb breed will influence tissue FA profiles and meat eating quality.

Chapter 6: In this chapter, the primary objective was to investigate the effects of graded levels of canola and flaxseed oil supplementation to purebred Merino and first-cross prime lambs on heart, liver and kidney FA profiles and plasma metabolites. The secondary aim was to evaluate the interactions of supplementation level with breed on these variables. This chapter tested the hypotheses that: (1) FA profiles of heart, liver and kidney, and plasma metabolites will be influenced by the oil supplementation and lamb breed, (2) there will be some effects of oil supplementation and lamb breed interaction on heart, liver and kidney FA profiles, and plasma metabolites.

Chapter 7: This chapter is a general discussion and conclusion of the main thesis outcomes and presentation of areas warranting further investigation.

Appendices: Contains all supplementary materials and copies of peer-reviewed publications from this thesis.

Chapter 2: Literature Review

2.1. Australian sheep industry background

2.1.1. Overview

The Australian sheep industry has undergone substantial change in both flock numbers and economic value. The national flock size has decreased from 138.2 million head in 1993 to 67.5 million head in 2016 (ABS, 2017a). Greasy wool production has drastically declined by approximately 60%, from 869 million kilograms in 1993 to 339 million kilograms in 2016 (AWI, 2017). As a consequence, the on-farm value of wool production has reduced from over $6 billion in 1992, to around $3.5 billion in 2017 (ABS, 2017b). In contrast, there has been a steady increase in lamb and mutton production. The real economic value of lamb and mutton production has substantially risen to around $3.6 billion in 2017 (ABS, 2017b). With decreasing wool production and the increasing prime lamb industry, the production of sheep meat has taken precedence over wool (ABS, 2017b).

Sheep production has shifted from predominantly wool-only farming to dual-purpose prime lamb systems. The prime lamb industry is becoming the key driver of the sheep industry with Merino ewes increasingly regarded as a 'maternal' breed (Rowe, 2010). This is a very significant change in focus for the sheep industry. The dual-purpose system requires more complex breeding and management decisions than a wool-only Merino production system. The dual-purpose system provides sheep breeders and producers with flexibility to shift their production system to suit prevailing economic forces dictated by dynamic consumer demands and market specifications (Kopke et al., 2008).

2.1.2. Wool industry

Sheep have been part of the Australian livestock production scene since the first fleet arrived in Botany Bay in 1788. In the first decades of the 20th century, Australia's wool exports accounted for three-quarters of all pastoral export incomes and prosperity in the wool industry peaked in 1951 (ABS, 2003). However, by 1971, wool production contributed only 15% to the total gross value of agricultural production (AG, 2015). Since 1987, the wool industry has had to face a sharp production decline and price reduction (Harle et al., 2007). Leith (2014) reported that Australian shorn wool production fell from 912,000 tonnes in 1991 to 360,000 tonnes in 2013. Despite the decrease in production and price, wool remains a substantial element of Australian sheep farm incomes. It is widely accepted that Australia produces the world's best quality woollen fibre: Australian merino wool. Australia produces over 50% of merino wool and over 90% of total superfine wool produced in the world (Swan, 2010). The reasons behind Australia's high quality wool can be predominantly attributed to the experience and expertise of Australian farmers in selecting superior animals for breeding purposes, and in using the harsh Australian climate to produce clean, fine wool of high strength.

The decline in Australian wool production is attributable to a number of factors: (1) strong competition from synthetic fibres, cotton and other natural fibres (Swan et al., 2007); (2) severe droughts and climate change that influence the quality of pastures, and the resistance to, and spread of, sheep diseases (Harle et al., 2007; Rowe, 2010; East and Foreman, 2011); (3) increasing lamb prices and greater returns from sheep meat production (East and Foreman, 2011); (4) the development of Chinese wool processing capacity (Barrett, 2012; Leith, 2014); (5) the collapse of the national Australian wool reserve price scheme in 1991 (Abbott, 2013); and (6) alternative land use pressures (Gleeson, 2013). However, against

these trends, Australian wool production increased by 4.3% during the 2016/2017 financial year and is forecasted to increase further by a modest 0.4% in the coming year (AWI, 2017). Australian Bureau of Agricultural and Resource Economics and Sciences (2017) stated that the price of wool, particularly ultrafine wool, had been increasing since 2014 and is still expected to keep at relatively high prices by the end of 2022. It also declared that wool demand is continuing to grow in China, which represents approximately 77% of Australian wool exports by volume. This is apparent in high clearance rates at Australian wool auctions, growing export value and growing demand for wool based products (Rural Bank, 2018). These robust market conditions appear to be encouraging producers to retain sheep for wool production.

2.1.3. Sheep meat production

The Australian sheep meat industry has delivered large increases in lamb production and profitability (Mortimer et al., 2014). The number of lambs slaughtered increased by 34% in the decade ending in 2016 to around 22.5 million head (MLA, 2017a). During a 6-year period, the number of sheep slaughtered increased from 4.9 million head in 2010 to 7 million in 2016 (MLA, 2017a). As a consequence, the economic value of lamb and mutton production substantially rose from $1.8 billion in 2009 (ABS, 2010) to approximately $3.2 billion in 2016 (ABS, 2017b). Rowe (2010) also stated that the focus on sheep meat production is now stronger than it has ever been in the history of the Australian sheep industry. This is likely due to a combination of strong demand for Australian lamb, especially in growing international markets (Thomas and Matthews, 2015), and genetic improvement to meet increasing consumer expectations (Fogarty, 2009). Another possible explanation for this increase lies in historically high prices for mutton and lambs that has occurred in the last decade (Martin, 2012).

Sheep meat accounts for only 3% of global meat production (Rowe, 2010). In 2015-16, Australia produced approximately 516,000 tonnes of lamb and 196,000 tonnes of mutton (MLA, 2017b). Australians consume on average around 9-11 kg of sheep meat products annually (McLeod et al., 2010; Thomas and Matthews, 2015). Approximately 47% of total lamb meat and 75% of mutton produced is exported (MLA, 2017b). Figure 2.1 shows that Australian sheep meat is mainly exported to traditional markets in the Middle East, the United States and to emerging markets in South-East Asia and China where there has been a huge growth in both population and income. Thus, both domestic and international markets have vast growth potential.

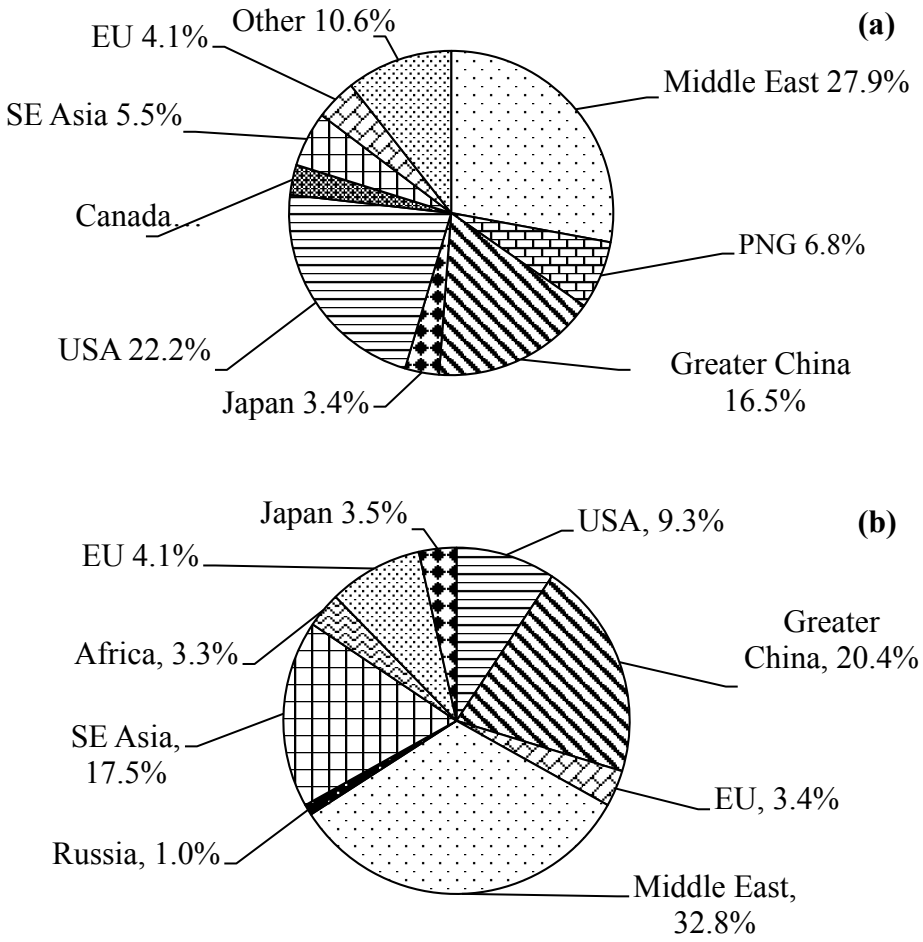

Figure 2.1. Australian exported markets of (a) 242,000 tonnes of lamb and (b) 148,000 tonnes of mutton in 2015-2016. Adapted from MLA (2017b).

2.1.4. Production trends

Increasing agricultural productivity and profitability continue to be core objectives of rural industries and Australian governments. In the sheep industry, as with multi-input, multi-output industries, the producer wishing to increase returns from a sheep enterprise must either reduce wool fibre diameter to maximise income from wool, or increase body weight to maximise income from meat (Adams and Cronjé, 2003). Therefore, it is essential to determine production trends based on available resources and consumer preferences. There have been several main production trends that are operating in the Australian sheep industry.

2.1.4.1. Self-replacing wool enterprises

Self-replacing wool enterprises account for 54% of the national flock, and on these farms, sheep purchases represent less than 10% of the average number of sheep in a flock (East and Foreman, 2011). These enterprises breed their own ewe replacements, and sell surplus young wethers and sheep culled for old age. Replacement rams are purchased to replace those culled for age. The producers in this sector are trying to select their sheep for finer wool fibre diameter (Rowe, 2010) and market surplus sheep sales into the more profitable lamb market.

Increasing demand for softer, lighter fabrics has resulted in alteration in wool fibre diameter (Nolan et al., 2014). Moreover, demand for Australian wool from China and some European countries has increased in recent years, particularly for fine (18.5-20.4 μm) and superfine wool (<18.5 μm) (Rees and Haylen, 2011).The prospect of earning more profitable returns from wool enterprises has led to a restructuring of the national flock with a trend to reduce fibre diameter. The production of superfine wool lifted from 3% to 26.8%

of the total Australian wool clip during the 1994-2014 period. In contrast, production of medium wool (20.5 - 24.4 μm) dropped by 42.6%. There has been a 3% increase in broad wool (⩾24.5 μm) over the same period (Figure 2.2). As a consequence, average fibre diameter has decreased from 22.4 μm to 21.4 μm between 1994 and 2016 (AWI, 2017). The small increase in the proportion of broad wool has been caused by an increase in crossbreeding for prime lamb production.

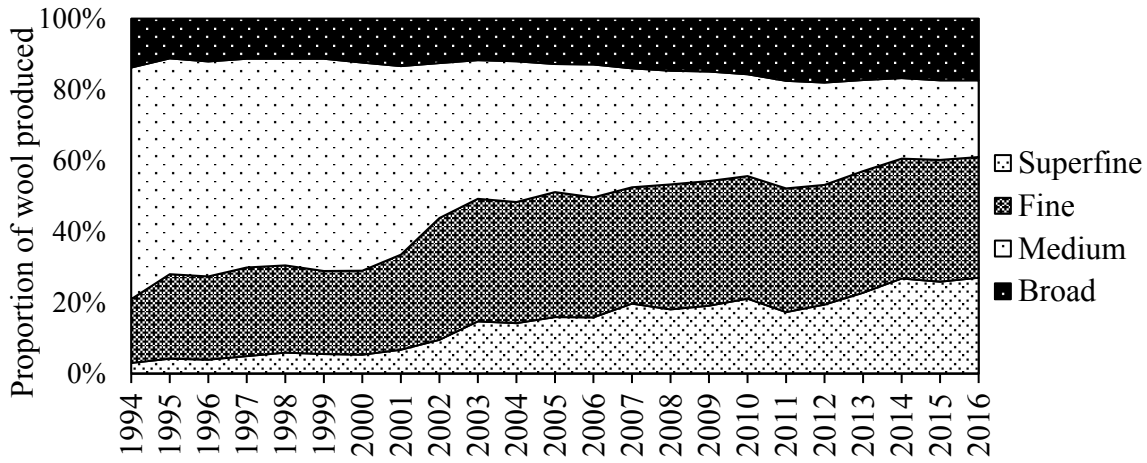

Figure 2.2. Trends in fibre diameter for Australian wool. Adapted from AWI (2017)

With the increasing proportion of Australian superfine wool, the demand for this wool in the retail woollen apparel industry is a major issue. Superfine wool is used to produce luxury apparel, but consumer demand is based on fashion value rather than functional quality (Rowe, 2010). Thus, economic prosperity is a key factor affecting demand for superfine wool. Gloomy economic growth has seen consumer confidence decline, with price and global demand for wool falling by 25% and 20% respectively from 1995 to 2008 (Gibbon and Nolan, 2011). However, a global shortage of wool supply is expected to improve prices. As key wool consuming countries recover, demand prospects are positive

for the Australian wool industry which supplies over 90% of global fine wool (Gibbon and Nolan, 2011).

2.1.4.2. Self-replacing meat farms

This sector represents 25% of the national flock and these farms derive greater than 50% of their total receipts from sheep and lamb sales (East and Foreman, 2011). This sector's emphasis is on carcass weight, with reduced focus on wool production. The farms generally use meat sheep breeds such as Corriedale, White Suffolk, Horned and Poll Dorset and Dorper. Meat sheep farmers supplement lambs with dietary concentrates during the finishing period in order to enhance growth rate and attain market specifications including carcass quality and fat score (Duddy et al., 2016). In 2009, almost 7% of meat sheep producers finished their lambs by feedlotting (ABARES, 2010).

Feedlotting is one of the feeding strategies which lamb producers frequently utilise during the finishing period to achieve a consistent supply of quality lamb. Its objective is to maximise growth rate, feed conversion efficiency and minimise the number of days on feed (Shirima et al., 2013). Use of feedlots allows producers to maintain production, when pasture availability is limited and income exceeds the cost of production. It also gives producers the flexibility to finish lambs irrespective of seasonal conditions (Duddy et al., 2016). In high rainfall grazing zones, processors are aligned with lamb breeders and finishers, thereby creating larger, more permanent feedlot enterprises. Such alliances provide stakeholders with opportunities to increase their profit by maximising stocking rates and supplying premium products to the market (AV, 2017). However, lamb feedlotting can be a high risk enterprise, with high economic technical input, but low benefits. Duddy et al. (2016) stated that feedlot enterprises involve financial risks, such as unexpected changes in market prices for lambs and feeds. Lamb deaths, shy feeders and

poor growth rates also are risk factors. Thus, producers who are feedlotting lambs need to be aware of the factors driving profitability in the system and implement strategies to minimise risk (MLA, 2007).

2.1.4.3. Dual-purpose lamb production

Many Australian sheep farmers are shifting from traditional wool-purpose only to dual-purpose production systems (Rowe, 2010; Malau-Aduli and Akuoch, 2012) to accommodate the increasing price and demand for prime lamb meat, and the decline in wool profitability because of stiff competition with wool from artificial fibres (Malau-Aduli et al., 2012a). Around 9% of the Australian flock is Merino-derived dual-purpose breeds. Dual-purpose production systems typically breed meat-type terminal sire breeds with a core flock of purebred Merino ewes (Malau-Aduli and Akuoch, 2012; Mortimer et al., 2017). It combines desirable meat and wool production traits within all progeny (Ingham et al., 2007). In addition, this tiered crossbreeding approach utilises individual heterosis and improves lamb tenderness, intramuscular fat level and growth (Mortimer et al., 2014). Hence, paternal meat and growth characteristics are inherited by crossbred progeny along with maternal Merino wool characteristics. However, maternal contributions are approximately 65% of the genes in progeny (Greeff et al., 2008). Paternal contributions also vary depending on sire breed selection. The meat-type terminal sire breeds used in Australian dual-purpose systems are Border Leicester, Dorset, Texel, White Suffolk, Hampshire Down and Ile De France (Pannier et al., 2014b). The most common crossbred ewe is the first-crossbred dual-purpose ewe which is usually the offspring of a long wool Border Leicester ram and a Merino ewe. The first-cross ewes are then mated to short wool meat breeds, such as the Dorset, Poll Dorset or Suffolk breeds, to arrive at the

Australian prime lamb. These first-cross ewes and their progeny comprise around 12% or more of the Australian sheep flock (Curtis, 2009).

The dual-purpose production systems have shared wool and meat market interests. Adams and Cronjé (2003) stated that if farmers attempt to balance risk by improving income from both meat and wool sources, economic profit will be less than when emphasis is on either attribute alone. Conversely, Kopke et al. (2008) reported that dual-purpose systems allow commodity diversification to provide a buffer against individual market volatilities rather than maintaining a sole focus on either prime lamb or wool. The dual-purpose enterprises produce more meat per hectare than the self-replacing wool enterprises and deliver greater wool incomes than the self-replacing meat enterprises (Warn et al., 2006). As a consequence, dual-purpose lamb producers can have a more profitable and flexible response in the sheep market. These systems appear to be relatively resilient to price changes, especially in unsustainable price scenarios and the decreasing availability of agricultural land.

2.2. Genetics management

Genetic progress is a key profit driver for the Australian sheep industry. Both the wool and prime lamb industries are interested in animals that have high carcass quality and excellent reproductive performance. Thus, there is considerable interest in breeding sheep for growth, meat, reproductive prolificacy, and wool quality traits (Brown and Swan, 2016). There has been significant industry investment in performance recording and genetic evaluation systems since the late 1980s (Swan et al., 2009) through MERINOSELECT for Merino sheep and LAMBPLAN for terminal and maternal sire breeds (Brown et al., 2007). Such progress has been driven by the significant shifts in the price relativities of meat and wool products in recent years (Safari et al., 2007).

Breeders and producers use a variety of breeds to take advantage of economically important traits such as daily growth rate, body weight, muscle development, carcass quality and wool fibre diameter. Numerous studies reaffirm that the Australian sheep industry is almost entirely based on Merino and its crossbreds (Greeff et al., 2008; Malau-Aduli and Akuoch, 2012; Wiedemann et al., 2015). The lamb industry uses purebred Merino and dual-purpose ewes such as Corriedale, Border Leicester and Coopworth for joining to terminal rams of various breeds that include Dorset and White Suffolk (Daetwyler et al., 2010). An overview of the use of MERINOSELECT and LAMBPLAN for managing wool sheep and prime lamb genetics will be covered in the next sections.

2.2.1. Wool sheep genetics management using MERINOSELECT

The Australian Merino originated from Spanish Merino, a Negretti strain, which was transported from England to Sydney, New South Wales in the early 1800s (Massy, 2007). Merino sheep have been one of the main pillars of the Australian economy for over 140 years and their history is inextricably linked with that of the nation (Massy, 2007) . The Australian Merino remains the dominant breed and plays a crucial role in the sheep industry. The pure Merino breed comprises over 60% of the Australian sheep population (MLA, 2017c). The breed is traditionally selected for breeding predominantly pure white wool characteristics (Greeff et al., 2008; Brown and Swan, 2016). The Australian Merino wool is widely regarded as the best quality wool in the world.

In a wide range of Australian environments and production systems, the breed has been broadly developed into three main types: fine wool sheep (Saxon and Spanish strains) suited to high rainfall zones; medium wools (Peppin strain) mainly run in cropping zones; and broad wools (South Australian strain) more common to the drier pastoral zones (Banks and Brown, 2009). Historically, commercial wool growers were supplied rams from stud

flocks characterised by a hierarchical breed structure (Parsons et al., 1996). However, this structure, with its high degree of relationship between animals, resulted in a high rate of inbreeding (Fogarty, 1978), which in turn reduced sheep performance and genetic diversity (Parsons et al., 1996; Swan et al., 2015). Since the use of artificial insemination became increasingly popular in the late 1980s, these stud flocks began to wane in influencing sheep breeding practices. The development stimulated many ram breeders to outsource new genetics from other flocks and participate in the national genetic evaluation system (with approximately 1.1 million sheep in 2006) known as, MERINOSELECT (Brown et al., 2006). MERINOSELECT was established to provide practical information on the genetic potential of rams for Merino breeders and wool growers (Brown et al., 2007). Sheep are ranked for various production characteristics using Australian Sheep Breeding Values (ASBVs). Flocks are directly compared through MERINOSELECT ASBVs, providing breeders with the opportunity to benchmark their sheep performance. This simplifies identification of the best sheep to meet breeding objectives. As a consequence, the Merino population has become increasingly admixed and the diversity between strains has been maintained (Swan et al., 2015).

During the two last decades, there have been strong premiums for finer diameter wool in the wool market, and Merino wool producers responded by achieving a finer wool from their flocks through a variety of means such as the nutritional management and selective breeding strategy. As a result, the average diameter of the Australian wool clip reduced by 1.4 μm between 1994 and 2016 (AWI, 2017). Merino flocks also act as a predominant genetic resource for both the prime lamb and mutton industries. Curtis and Croker (2005) estimated that one quarter of Merino ewes were mated to rams from meat-type breeds to generate progeny with high value carcasses. Greeff et al. (2008) stated that Merino

contributes over 65% of the genes in slaughter lambs largely through crossbreeding. The breed dominates 99.9% of mutton production (Connell and Hooper, 2001).

2.2.2. Crossbred prime lamb genetics management using LAMBPLAN

Crossbreeding has traditionally been used in the Australian prime lamb industry to select animals for faster growth, improved muscling and reduced fat (Brown and Swan, 2016). It is based on a biologically sensible structure: terminal sire breed rams are mated to either first-cross Merino or purebred ewes such as Corriedale or Merino (Banks, 1990). This system takes advantage of hybrid vigour from the maternal breeds (mothering and reproductive abilities), and from terminal rams (muscle production and growth performance) (Daetwyler et al., 2010; Malau-Aduli et al., 2012b). Annett et al. (2011) stated that crossbreeding provides opportunities to introduce complementary traits of lamb growth and prolificacy, and the exploitation of heterosis for reproduction, survival and body conformation traits.

In the late 1960s, genetic recording programs to assist Australian sheep breeders were first developed in New South Wales, South Australia and Victoria (Pattie, 1973). Such programs demonstrated that genetic improvement plays a key role in contributing to the enhancement of economically important traits, although they were unsuccessful in attracting breeders. However, in the late 1970s, sheep breeders showed renewed interest in genetic programs and realised the importance of genetics in the development of the industry. Beginning with growth and fatness in Dorset production competitions in Cowra (Clements and Fogarty, 1976), several research and development programs were undertaken in the early 1980s. For instance, Meat Sheep Testing Service implemented a widespread recording program with Dorset and other terminal sire breeders in New South Wales and its recording expanded to over 17,000 sheep annually (Harris, 1985). Furthermore,

consumer research was undertaken in the late 1970s and early 1980s to identify an overall strategy to develop the prime lamb industry that could meet product specifications of lucrative markets. In 1984, the introduction of real-time ultrasonic equipment was a major development for the livestock industry, as it provided accurate measurements of fat depth in live animals (Stouffer, 2004). As a result, Meat and Livestock Australia sponsored a research and development project to evaluate growth rate and fat depth of rams from different genetics (Fogarty, 2009). The project developed practical procedures for measuring fat depth using real-time ultrasound technology, accumulated a large database and provided a model for the development of LAMBPLAN.

LAMBPLAN, the national genetic evaluation program, was established in 1989 to provide practical information on the genetic potential of breeding rams for the prime lamb industry (Banks, 1990). LAMBPLAN utilises the Best Linear Unbiased Prediction (BLUP) algorithms to predict the ability of an individual ram to produce superior progeny (Gilmour and Banks, 1992). The BLUP algorithms analyse data relating to not only the ram's own performance, but also the performance of all recorded relatives in the flock, which is recorded on a national database. The trait data that is recorded on the database is collected by accredited LAMBPLAN operators and captured using various accurate trait measurement technologies such as real-time ultrasound. The ram and its progeny performances are articulated in the form of Estimated Breeding Values (EBVs) and Indexes that combine EBVs for specific meat production and reproductive traits. Effectively, EBVs allow ram buyers to select terminal rams for breeding with either purebred ewes or first-cross ewes to produce prime lambs. EBVs are also employed to choose maternal rams that are crossed with Merino ewes to produce first-cross ewes that meet the producer's reproduction efficiency objectives. In general, animal measurement and data processing are

performed on-farm on the same day (Banks, 1990). The introduction of LAMBPLAN has resulted in a number of significant benefits: (1) reducing risks related to breeding stock selection and improving animal productivity; (2) the genetic achievement of involved flocks can be tracked through LAMBPLAN benchmarking system; (3) LAMBPLAN provides breeders with flexibility to focus on important traits which meet breeding objectives and market required specifications; and (4) producers can objectively compare and choose the optimal rams for their production system and market targets (Banks, 2000).

2.2.3. Sheep Genetics Australia

Historically, sheep genetic data in separate databases were evaluated by different organisations with various models, and for different objectives. In addition, the results of these genetic evaluations were often presented in varying forms to both ram breeders and commercial sheep producers (Brown et al., 2006). Sheep Genetics Australia was established in 2005 to deliver a nationally consistent across flock genetic evaluation platform to the Australian sheep industry, generating information relevant for terminal, maternal and Merino sheep (Brown et al., 2007). The main purpose of SGA is to improve the quality, scope and utilisation of across-flock genetic information for the Australian sheep industry, using a common national language in the form of ASBVs.

Sheep Genetics Australia currently runs the largest sheep genetics database in the world and uses it to calculate "credible and accurate" breeding values (Lupton, 2008). It runs two major selection programs (LAMBPLAN and MERINOSELECT). Sheep Genetics Australia also delivers the genetic evaluation for the Dohne Merino breed and KIDPLAN, a goat industry service (AVC, 2016). In the first decade, it was a joint program of Australian Wool Innovation (AWI) and Meat and Livestock Australia (MLA). LAMBPLAN was 100% owned and funded by MLA. Ownership of MERINOSELECT was split 50/50 between the

two organisations. Since July 2016, both LAMBPLAN and MERINOSELECT have been owned and funded by MLA (AVC, 2016).

The genetic evaluation services provided by SGA underpin the breeding decisions that drive genetic gain in nucleus flocks which then flows through to commercial flocks (Walkom and Brown, 2017). Unlike many other farm inputs, genetic superiority is permanent and cumulative, meaning that improvements will be transmitted to subsequent generations without further endeavour or expenditure (Van Eenennaam, 2013). Sheep Genetics Australia services have been improved and have built upon the outcomes of many years of research, much of it funded by the sheep industry. Due to more recent advances from molecular genetics or genomics, quantitative genetics is gradually being supplemented. Consequently, the accuracy of genetic evaluations increased further and the progress is faster (AVC, 2016).

2.3. Omega-3 polyunsaturated fatty acids: health benefits and sources

2.3.1. The molecular structures of omega-3 polyunsaturated fatty acids

Fatty acids are typically named with reference to the number of carbon atoms in the hydrocarbon chain (Ruxton et al., 2004). Saturated fatty acids only have single bonds between adjacent carbon atoms, while FA with at least one double bond in their structure are referred to as unsaturated. Unsaturated fatty acids (UFA) are divided into the monounsaturated fatty acids (MUFA) and PUFA on the basis of the number of double bonds (Lorente-Cebrián et al., 2013).

Table 2.1. List of omega-3 fatty acids

Common name	Abbreviation	Number of carbon atoms	Number of double bonds	Symbol
Hexadecatrienoic acid	HTA	16	3	16:3 n-3
Alpha linolenic acid	ALA	18	3	18:3 n-3
Stearidonic acid	SDA	18	4	18:4 n-3
Eicosatrienoic acid	ETE	20	3	20:3 n-3
Eicosatetraenoic acid	ETA	20	4	20:4 n-3
Eicosapentaenoic acid	EPA	20	5	20:5 n-3
Heneicosapentaenoic acid	HPA	21	5	21:5 n-3
Docosapentaenoic acid	DPA	22	5	22:5 n-3
Docosahexaenoic acid	DHA	22	6	22:6 n-3
Tetracosapentaenoic acid	TPA	24	5	24:5 n-3
Tetracosahexaenoic acid	THA	24	6	24:6 n-3

An omega-3 polyunsaturated fatty acid is an UFA with multiple double bonds. The first double bond in n-3 PUFA is located between the third and fourth carbons, counting from the methyl (CH_3) end of the molecule (Swanson et al., 2012). In nature, 11 different n-3 PUFA have been found and they are listed in Table 2.1.

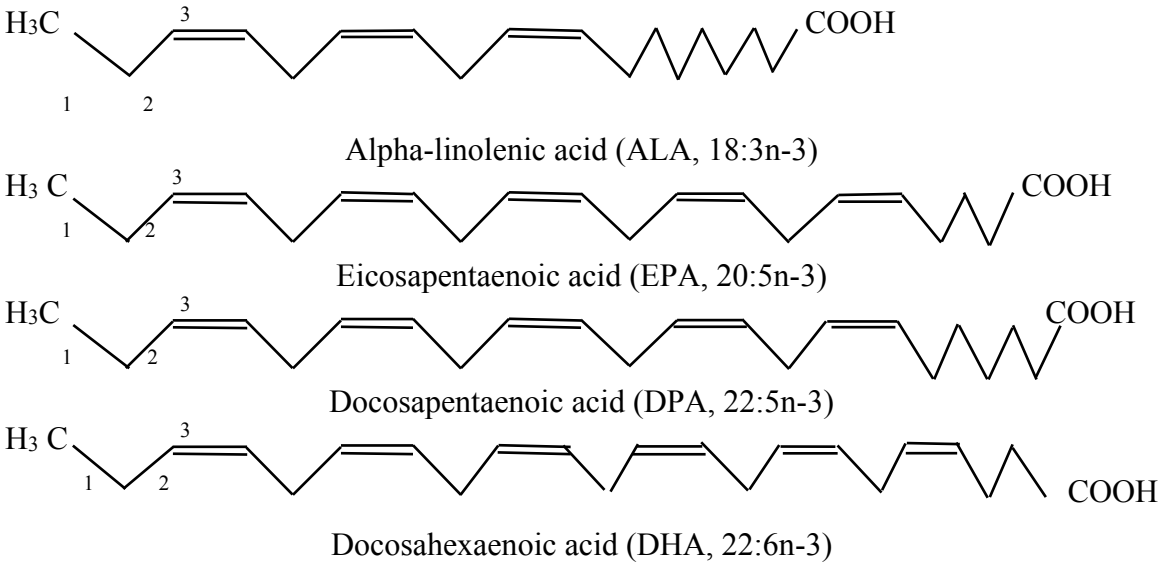

Figure 2.3. The structure of ALA, EPA, DPA and DHA showing the position of the first double bond between the third and fourth carbons from the methyl end.

Four n-3 PUFA, namely alpha-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are very important in human physiology and health (Nichols et al., 2014). The chemical structures of these four main n-3 PUFA are presented in Figure 2.3.

2.3.2. Human health benefits

Fat consumption is essential for human development, health, and longevity (Gropper and Smith, 2013). Omega-3 long-chain (containing 20 or more carbon atoms) PUFA (LC-PUFA) are integral components of all cell membranes in the body as either membrane phospholipids or free molecules (Gorjão et al., 2009; Khan and He, 2017). They contribute considerably to the physical properties of biological membranes, including membrane organization, ion permeability, elasticity and microdomain formation (Gorjão et al., 2009). Ganesan et al. (2014) reported that when released from the cell membranes, the n-3 LC-PUFA become the precursors of eicosanoid hormones such as resolvins (products from EPA) and docosatrienes, protectins, and neuroprotectins (products from DHA) which are important in the defence against, and treatment of various diseases (Calder, 2006; Khanapure et al., 2007).

The broad health benefits of n-3 LC-PUFA in preventing many diseases have been well documented (Ruxton et al., 2004; Swanson et al., 2012; Nichols et al., 2014). DHA is present in large amounts in brain and retina membrane phospholipids (Swanson et al., 2012; Walker et al., 2015). Innis (2008) and Gould et al. (2013) indicated that n-3 LC-PUFA play a fundamental role in neural development in embryos. Increasing n-3 LC-PUFA intake has been associated with reduced risk of dementia and delayed decline in cognitive function (Ruxton et al., 2004; Swanson et al., 2012; Khan and He, 2017). Increased intake of n-3 LC-PUFA results in increased amounts of n-3 LC-PUFA and decreased amounts of

arachidonic acid in the phospholipids of inflammatory cells (Calder, 2015). This leads to a decrease in the production of inflammatory mediators such as arachidonic acid–derived eicosanoids and cytokines. These mediators act both directly, by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism, and indirectly, by reducing the expression of inflammatory genes through effects on transcription factor activation (Calder, 2006). Furthermore, n-3 LC-PUFA are also substrates for anti-inflammatory mediators (Calder, 2013). Thus, many studies in both animals and humans have demonstrated that n-3 LC-PUFA are potential therapeutic agents for suppressing inflammation and thereby have a beneficial role in a variety of inflammatory diseases including diabetes, atherosclerosis, asthma and arthritis (Calder, 2006, 2013, 2015; Simopoulos, 2016).

Cardiovascular diseases (CVD) and cancer are the leading causes of death worldwide (Siegel et al., 2016; Benjamin et al., 2017). The cardio-protective effect of PUFA was first postulated in the 1950s (Sinclair, 1956). Scientists observed that Alaskan and Greenlandic Eskimos and Okinawa islanders had a reduced incidence of CVD and other chronic diseases than other groups because of their high consumption of fish and marine mammals, both being rich in n-3 LC-PUFA (Bang et al., 1980; Kagawa et al., 1982). Nowadays, many published studies have comprehensively established and highlighted that n-3 LC-PUFA play a critical role in the prevention of CVD in humans (Nichols et al., 2014; Calder, 2017; Watanabe and Tatsuno, 2017). The consumption of n-3 LC-PUFA may reduce the risk of CVD by reducing systolic resting heart rate (Mozaffarian et al., 2005), lowering blood viscosity (Cartwright et al., 1985), inhibiting platelet aggregation (Simopoulos, 2002), improving blood vessel function (Abeywardena and Head, 2001) and reducing plasma fibrinogen (Watanabe and Tatsuno, 2017). Simopoulos (2002) stated that n-3 LC-PUFA,

when supplied in high doses, reduce plasma cholesterol and have anti-thrombotic and hypotriglyceridemic properties. The beneficial effects of long-term intake of n-3 LC-PUFA on cancer patients have also received intense attention by both clinicians and epidemiologists (Berquin et al., 2008; Laviano et al., 2013). Based on epidemiological studies, Rose and Connolly (1999) and MacLean et al. (2006) suggested that people whose diets are high in n-3 LC-PUFA, may experience a lower incidence of common cancers such as breast, colon, and prostate. Many mechanisms are involved, including elimination of neoplastic transformation, inhibition of cancer cell growth (Heller et al., 2004), and enhanced apoptosis and anti-angiogenicity, through the prevention of eicosanoid production from arachidonic acid precursors (Rose and Connolly, 1999). Berquin et al. (2008) added that n-3 LC-PUFA can serve as a nutritional source for cancer patients to reduce weight loss, enhance recovery after surgery and modulate the immune system.

As an individual n-3 LC-PUFA, the less studied DPA also has positive correlations with lower incidence of CVD and cancers, mental health and inflammatory disorders (Kaur et al., 2011; Byelashov et al., 2015). Furthermore, epidemiological studies have demonstrated that DPA consumption has some unique and specific benefits in human health nutrition: (1) DPA prevents platelet aggregation more efficiently than EPA and DHA (Phang et al., 2009); (2) DPA may act as a precursor for production of the DPA-related D-series of resolvins or neuroprotective compounds (Kaur et al., 2011); (3) DPA is a potent stimulator of endothelial cell migration, which is an essential component of embryonic vascular system (Aase et al., 2007) and it acts much more efficiently than EPA (Kaur et al., 2016); (4) DPA is also incorporated into cell phospholipids faster than EPA (Byelashov et al., 2015); and (5) DPA has more potent anti-proliferative and pro-apoptotic effects on cancer cells than both EPA and DHA (Morin et al., 2013).

Furthermore, DPA can serve as an intermediate reservoir contributing to the biosynthesis of both EPA- and DHA-derived bioactive lipid mediators (Miller et al., 2013; Markworth et al., 2016). Supplementing young female adults with 8 g of pure DPA over a 7-day period, Miller et al. (2013) found that the levels of not only DPA but also both EPA and DHA in plasma triacylglycerol (TAG) fractions were increased. The increases in all three n-3 LC-PUFA indicate that DPA is both being further desaturated to DHA and retro-converted back to EPA. Retro-conversion involves both peroxisomal acyl-CoA oxidase and β-oxidation (Christensen et al., 1993). The retro-conversion has been observed in human fibroblasts (Rosenthal et al., 1991; Christensen et al., 1993). The evidence of retro-conversion of DPA to EPA was found in various animal tissues including rat liver, heart and skeletal muscle (Kaur et al., 2010; Holub et al., 2011), and bovine endothelial cells (Achard et al., 1995). The increase in DHA levels in plasma TAG fractions in rat liver following pure DPA supplementation was also reported by Holub et al. (2011) and Kaur et al. (2010). These findings demonstrate that DPA can act as a source of DHA in the body. However all of aforementioned studies could not clearly describe the desaturation mechanism of DPA to DHA. Markworth et al. (2016) stated that the metabolic fate and novel mechanism that may explain bioactivity of DPA have rarely been investigated. The role of DPA in human health has been largely ignored (Kaur et al., 2011), and knowledge of its beneficial impacts on animal nutrition remains limited (Alvarenga et al., 2015). Hence, more research is required determine the biological effects and importance of DPA in both animal nutrition and human health, and to systematically investigate its metabolic fate and physiological mechanism.

2.3.3. Dietary sources and intakes

The n-3 PUFA are considered essential because they cannot be synthesized by the human body and other vertebrates due to the lack of Δ-12 and Δ-15 desaturase enzymes (Alvarenga et al., 2015) which can form carbon–carbon double bonds beyond the Δ-12 and Δ-15 carbons (Innis, 2008). Therefore, the n-3 PUFA need to be acquired through dietary sources (Rose and Connolly, 1999; Simopoulos, 2016).

In the human body, omega-3 medium-chain (C_{18}) PUFA can be converted by elongation and desaturation to more unsaturated and n-3 LC-PUFA, which are more bioactive than their precursors. Alpha-linolenic acid is converted via several intermediates to EPA, which is further elongated to DPA and DHA by the pathway shown in Figure 2.4. The conversion of ingested ALA to n-3 LC-PUFA within the body is not usually considered to be a reliable source of n-3 LC-PUFA in the human diet. In healthy adults, conversion rates of dietary ALA to EPA and DHA are almost 6% and 3.8% respectively (Gerster, 1998). Burdge et al. (2002) showed that, only about 8% of ALA in young men diets is converted to EPA and DPA, and none is converted to DHA. The possible explanations for the inefficient conversions are that most of dietary ALA are utilised as energy sources (Woods and Fearon, 2009) and diets are rich in omega-6 PUFA (Gerster, 1998). Hence, it is more beneficial and practical to directly consume n-3 LC-PUFA from daily food as preformed EPA, DPA and DHA, rather than as ALA.

There are many dietary sources of n-3 PUFA including seafood, animal products and land plants. The type and amount of n-3 PUFA varies in the many sources. Alpha-linolenic acid is mostly found in the chloroplast of green leafy plants, some oilseeds, and in vegetable oils such as flaxseed and canola (Deckelbaum and Torrejon, 2012; Baker et al., 2016). Eicosapentaenoic acid and DHA are found in significant amounts in seafood, especially fish oils (Calder, 2015; Nichols et al., 2016; Calder, 2017).

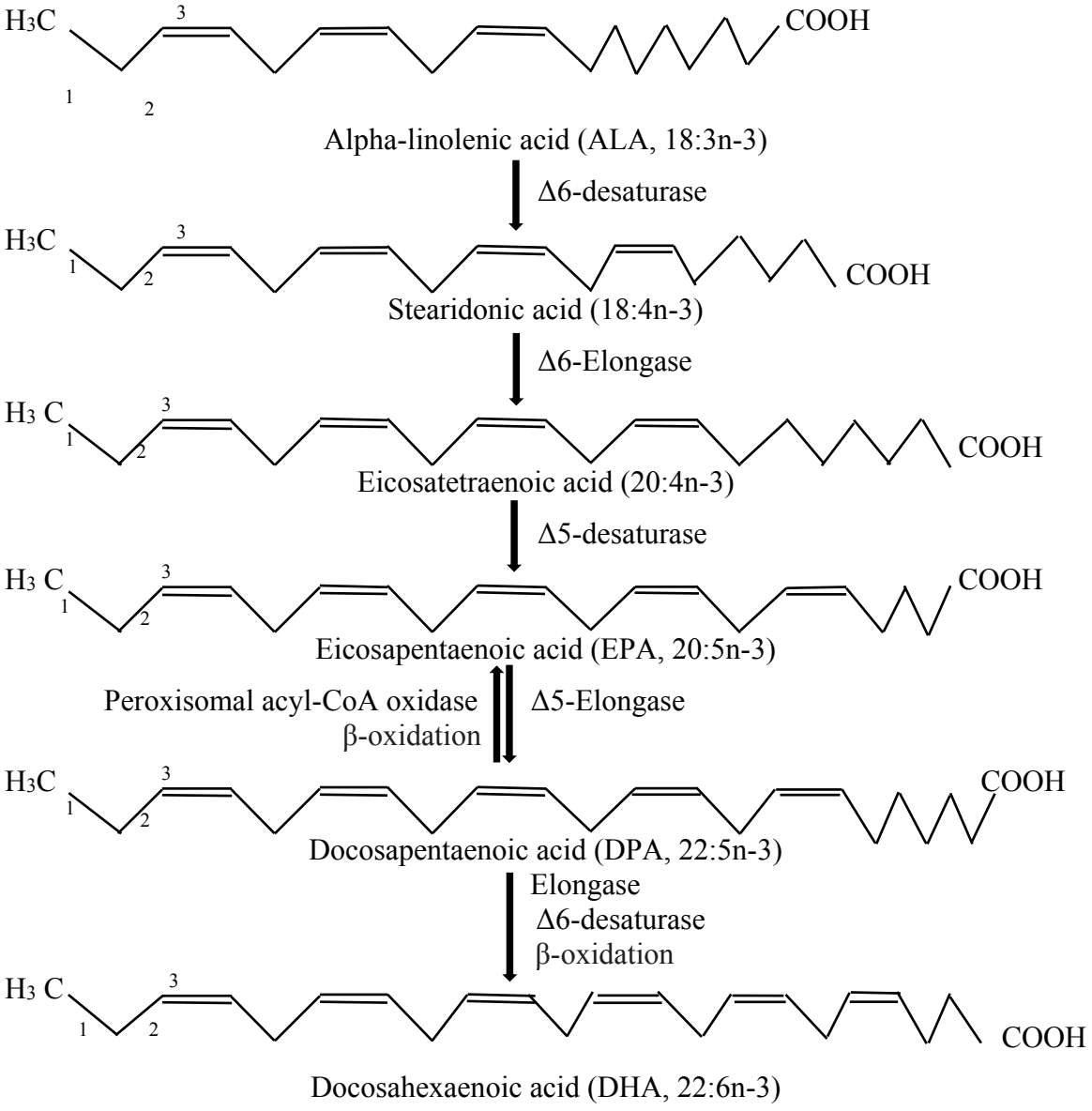

Figure 2.4. A general pathway for desaturation and chain elongation of omega-3 polyunsaturated fatty acids. Adapted from Calder (2017).

Although, seafood also is the richest source of DPA, red meat appears to be a main dietary source of DPA and a major source of n-3 LC-PUFA in some countries such as Australia and the United State (Clayton, 2014; Byelashov et al., 2015). In Australia, the largest

dietary contributors of DPA in children are red meat, poultry and game products (56%), followed by seafood (23%) (Rahmawaty et al., 2013). The content of DPA in red meat is relatively higher than those of EPA and DHA. Droulez et al. (2006) reported that DPA content in the lean component of Australian red meat cuts varied from 32 mg/100g to 54 mg/100g, whereas the contents of EPA and DHA in the same cuts ranged from 29 mg/100g to 46 mg/100g and from 6 mg/100g to 19 mg/100g respectively.

Due to the potential health benefits, the recommended dietary n-3 LC-PUFA intakes have been published by many organisations. Table 2.2 summarises the recommendations for n-3 LC-PUFA consumption from several associations and organisations. In general, consumption of two to three servings per week of oily fish rich in n-3 LC-PUFA is suggested to provide about 500 mg of EPA+DHA daily, for primary prevention of CVD (Kris-Etherton and Innis, 2007; FFSA, 2010; Nichols et al., 2010; NHFA, 2015). However, it should be noted that different amounts of n-3 LC-PUFA are suggested for males and females (NHMRC, 2006). The American Heart Association recommended greater consumption of n-3 LC-PUFA for people with specific diseases. Patients with documented coronary heart disease should consume approximately 1000 mg of n-3 LC-PUFA from oily fish or fish oil capsules. For patients with severe hypertriglyceridemia, who want to lower triglyceride levels, the recommended effective doses of n-3 LC-PUFA are from 2000 to 4000 mg per day (Miller et al., 2011).

Table 2.2. Recommended weekly fish and/or daily omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) intakes from health organisations

Organisation	Focus	Recommendation		Reference
		Weekly fish meal (112 g/serving)	Daily n−3 LC-PUFA (mg/day)	
American Heart Association	Coronary heart disease (CHD) sufferers Individuals with hypertriglyceridemia	2 servings	1000 2000-4000	Miller et al., (2011)
FAO/WHO Expert Consultation	For secondary prevention of CHD		250-2000 of EPA+DHA	FAO/WHO (2008)
American Diabetes Association	For primary prevention of CHD	at least 2 servings		Bantle et al. (2008)
Japanese Ministry of Health, Labour and Welfare (JMHLW)	Individuals over the age of 2		>1000	JMHLW (2015)
American Dietetic Association and Dietitians of Canada	For primary prevention of CHD	2 servings	500 of EPA+DHA	Kris-Etherton and Innis (2007)
Academy of Nutrition and Dietetics	All adults		500 of EPA+DHA	Vannice and Rasmussen (2014)
European Food Safety Authority	All adults	1-2 servings	250 of EPA+DHA	Tur et al. (2012)
French Food Safety Agency (FSSA)	Individuals over the age of 10	2 servings	500 of EPA+DHA	FFSA (2010)
National Heart Foundation Australia (NHFA)	CHD sufferers	2-3 servings	250-500 of EPA+DHA	NHFA (2015)
Australia New Zealand National Health and Medical Research Council (NHMRC)	Healthy female adults Healthy male adults		430 610	NHMRC (2006)

FAO/WHO: Food and Agriculture Organization /World Health Organization

In Western countries, various studies have agreed that median levels of n-3 LC-PUFA consumption are insufficient (Byelashov et al., 2015; Walker et al., 2015). Americans currently consume 99 g of seafood per week with considerably less EPA+DHA than the recommended values (Papanikolaou et al., 2014; Walker et al., 2015). In Australia, Birch and Lawley (2014) showed that the weekly consumption of seafood has slowly increased to approximately 220 g per capita in 2011. However, recent studies on consumption reported that Australians are not consuming recommended quantities of n-3 LC-PUFA (Rahmawaty et al., 2013; Fayet-Moore et al., 2015). Howe et al. (2006) estimated that the average n-3 LC-PUFA intake in Australia was 246 mg/day. Hence, it is necessary to explore more practical options of achieving the n-3 LC-PUFA recommendations.

The low consumption of n-3 LC-PUFA is due to numerous factors. Fishy taste and high cost of seafood are commonly cited reasons for the low consumption of n-3 LC-PUFA (Kennedy et al., 2012). Additionally, many people rarely eat fish because of its low availability in many geographical locations (Walker et al., 2015). Birch and Lawley (2014) pointed out that habit, such as regular childhood consumption and seafood familiarity, also influence seafood consumption. The bioaccumulation of toxic contaminants such as mercury, arsenic and lead in fish, is another issue reducing fish consumption (Bosch et al., 2016; Gribble et al., 2016). Other concerns are overfishing and growing global population that could strain the sustainability of the market (Kennedy et al., 2012).

The content of DPA in human milk was higher than that of EPA and similar to DHA content (Koletzko et al., 1988), indicating it potentially plays an important role in infant development. The potential health benefits of DPA is currently emerging (Kaur et al., 2016; Calder, 2017). However, many health organisations worldwide only offer guidelines for n-3 LC-PUFA intake without DPA (Byelashov et al., 2015). Howe et al. (2006) reported that DPA may contribute almost one-third of total n-3 LC-PUFA in Australian diets. Vahmani

et al. (2015) stated that the exclusion of DPA from total n-3 LC-PUFA intake results in reducing the total amount of their actual consumption. Thus, the development of alternative approaches of incorporating n-3 LC-PUFA into the human diet are required. Currently, the enhancement of n-3 LC-PUFA content in sheep meat as an alternative source for human consumption has gained significant research attention (Howes et al., 2015; Flakemore et al., 2017; Nguyen et al., 2017).

2.4. Metabolism of omega-3 PUFA in ruminants

The nature of lipid digestion by animals has a substantial effect on the transfer of fatty acids from the diet into tissue (Woods and Fearon, 2009). Once dietary lipids enter the rumen, the microbes are thought to be primarily responsible for transforming lipids via two major processes, namely lipolysis and biohydrogenation (BH) (Jenkins et al., 2008; Buccioni et al., 2012; Edwards et al., 2017). The initial stages of lipid digestion are characterised by intense lipolysis. Shortly after esterified dietary lipids are consumed, more than 85% of galactolipids, phospholipids and triglycerides are hydrolysed by microbial lipases to release non-esterified fatty acids including UFA (Buccioni et al., 2012; Shingfield et al., 2013). After lipolysis, UFA undergo BH by the rumen microbes (Jenkins, 1993). The process involves the removal of double bonds through microbial enzyme activity and by using hydrogen which is provided from dietary fermentation in the rumen. During BH, PUFA are converted into MUFA and ultimately to SFA with a vast array of *trans* FA intermediates and isomers being formed simultaneously (Harfoot and Hazelwood, 1998; Buccioni et al., 2012). Many studies have concluded that ruminal BH is one of the main challenges working against attempts to increase n-3 LC-PUFA in ruminant tissues through dietary supplementation (Bessa et al., 2015; Howes et al., 2015; Alves et al., 2017).

The BH of dietary n-3 PUFA in the rumen is slightly complicated compared to other common UFA. The greater degree of unsaturation of n-3 PUFA requires a greater species

diversity of ruminal microbes (Lourenço et al., 2010). The BH of these FA also involve numerous steps of isomerization, hydrogenation of double bonds and chain shortening (Jenkins et al., 2008). As a consequence, the BH of n-3 PUFA can produce a wide range of UFA intermediates and final SFA products (Shingfield et al., 2012). The pathways of ALA biohydrogenation are illustrated in Figure 2.5. The products of ALA during the ruminal BH are: rumelenic acid, 18:2 geometric isomers, 18:1 geometric isomers, vaccenic acid, and stearic acid. Doreau and Ferlay (1994) and Glasser et al. (2008) concluded that greater than 85% of dietary ALA is hydrogenated in the rumen. Several studies have reported that EPA and DHA are extensively hydrogenated in the rumen *in vivo* (Shingfield et al., 2012) and disappear during *in vitro* incubation with mixed ruminal microorganisms (Kairenius et al., 2011).

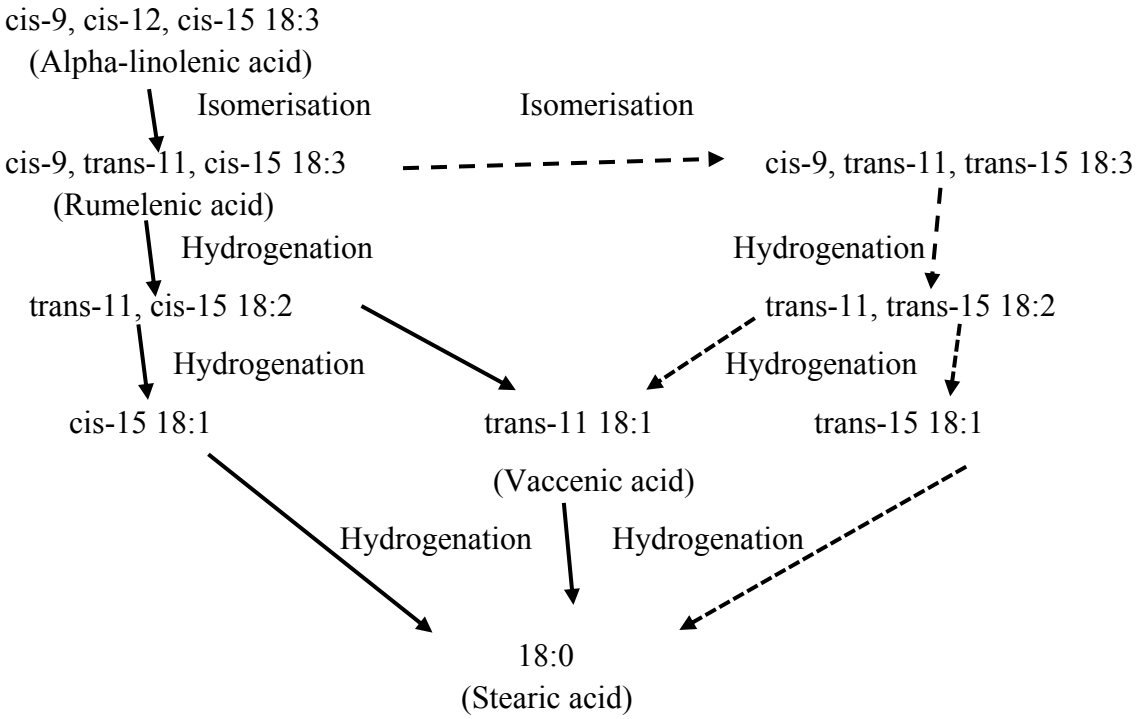

Figure 2.5. Ruminal biohydrogenation pathways of alpha-linolenic acid. Adapted from Gómez-Cortés et al. (2009)

Shingfield et al. (2010) also observed more than 90% of dietary EPA and DHA from fish oil are not recovered in the duodenum, although when supplemented as algae products they

might be much less hydrogenated (60%) (Sinclair et al., 2005). Chilliard et al. (2007) and Shingfield et al. (2012) stated that the large extent of EPA and DHA biohydrogenation results in a wide array of intermediates including large amount of UFA and a small number of SFA. The presence of these FA inhibits the BH of ALA (Shingfield et al., 2010). The extensive BH of n-3 PUFA occurs because of the toxicity of PUFA to rumen bacteria namely those related to *Butyrivibrio* (Sakurama et al., 2014). However, the pathways for the BH of EPA, DPA and DHA in the rumen have not been studied in detail (Sakurama et al., 2014; Alvarenga et al., 2015). Further studies are required to clearly characterise the BH pathways of these n-3 LC-PUFA and their products.

Several factors have been reported to modulate the BH of dietary n-3 PUFA, such as the amount and type of the lipid supplements, the basal diet and duration of feeding (Shingfield et al., 2013; Chikwanha et al., 2017; Realini et al., 2017). The use of secondary plant metabolites such as tannin, saponins and essential oils (Willems et al., 2014; Girard et al., 2016; Alves et al., 2017), and/or the supplementation of concentrate diets with rich sources in n-3 PUFA has been shown to reduce the ruminal BH rate and significantly improve the sensory and nutritional quality of lamb (Ponnampalam et al., 2016a; Realini et al., 2017). Other studies minimised the BH rate by using herbage (Buccioni et al., 2012) or changing ruminal pH (Lascano et al., 2016). In a review article, Nudda et al. (2014) stated that retention times or passage rates of feed from the rumen influence the extent and completion of BH. Sheep have a smaller rumen than cattle which reduces retention times and therefore results in BH being less complete in sheep compared to cattle. Consequently, this increases duodenal flow, absorption and deposition of n-3 PUFA and BH intermediates into tissues in sheep (Chikwanha et al., 2017).

The lipids of post-ruminal digestion includes mainly SFA (stearic acid and palmitic acid), BH intermediates and microbial phospholipids, along with dietary by-pass triglycerides (Chilliard et al., 2007; Alvarenga et al., 2015). The absorption of UFA into the small intestine by ruminants is similar to that in monogastric animals (Woods and Fearon, 2009). The intestinal uptake coefficient of PUFA is up to 92% for conventional low fat diets and is greater in ruminants compared to monogastric animals (Doreau and Ferlay, 1994). Doreau and Ferlay (1994) also stated that there is a decrease in FA digestibility in the small intestine when fat intake increases. The apparent digestibility coefficients are on average 70% for n-3 PUFA (Glasser et al., 2008; Doreau et al., 2016). However, small amounts of n-3 LC-PUFA are incorporated into triglycerides in adipocytes. They are mainly incorporated into the membrane phospholipids and are deposited in significant amounts in intramuscular tissue (De Smet et al., 2004; Alvarenga et al., 2015). Lean meat per gram of tissue is richer in n-3 LC-PUFA compared to fattier meat and adipose tissues.

2.5. Omega-3 PUFA sources for ruminants

Lipid in ruminant diets is very important, not only due to its significant energy contribution, but also because it supplies essential FA and fat-soluble vitamins (Woods and Fearon, 2009). The decision to use fat or oil and the form in which it is included in the feed is influenced by a number of factors. These factors include: (1) the composition of basal diets; (2) lipid form (whole oilseed, processed oilseed or extracted oil) and its digestibility (Shingfield et al., 2013; Gómez-Cortés et al., 2014; Ponnampalam et al., 2015); (3) the price and availability of raw material; and (4) feed supply permits and animal welfare regulations (Woods and Fearon, 2009). Since dietary lipid is one of major factors affecting the FA profile of ruminants (De Smet et al., 2004), enrichment of red meat with health benefitting n-3 PUFA can be attained by innovative nutritional approaches (Doreau et al.,

2016; Ponnampalam et al., 2016a; Chikwanha et al., 2017). The main n-3 PUFA sources supplied in ruminant diets include forage sources, fish oils and marine products, oilseed, and terrestrial plant oils (Woods and Fearon, 2009; Scollan et al., 2014; Alvarenga et al., 2015).

2.5.1. Forage

In general, consumed forage is the main source of n-3 PUFA for ruminants although the amounts of n-3 PUFA consumed varies with several factors. For example, fresh grass or pasture has greater n-3 PUFA content than silage and hay (Scollan et al., 2014; Alvarenga et al., 2015). Fresh grass is not a source of EPA and DHA, but is rich in ALA localised in the chloroplasts, accounting for over 50% of total FA (Dewhurst et al., 2006; Chilliard et al., 2007; Wood et al., 2008). Variations in grass FA profile during ensiling may be due to microbial intervention, which results in hydrogenation and isomerization similar to that which occurs by microbial action in the rumen (Alves et al., 2011). Dewhurst et al. (2006) and Kalač and Samková (2010) observed that field wilting prior to ensiling and drying during hay making can result in major losses of ALA due to oxidation. Glasser et al. (2013) concluded that ALA content of alfalfa hay is almost half that of fresh alfalfa. The ALA content of grass can also be influenced by variety and maturity (Woods and Fearon, 2009; Glasser et al., 2013). Girard et al. (2016) determined that alfalfa silage had a lower ALA proportion than red clover and sainfoin silages. Furthermore, Koivunen et al. (2015) indicated that within the same variety, advancing maturity reduced ALA content of Timothy–meadow fescue grass and red clover.

A number of studies have demonstrated that n−3 PUFA content in red meat and cow milk are greater in animals fed pasture-based compared to concentrate-based diets (Shingfield et al., 2013; Vazirigohar et al., 2014; Howes et al., 2015; Jaturasitha et al., 2016). Eriksson

and Pickova (2007) suggested that fresh grass exerts a greater protection for n-3 PUFA against rumen microorganisms than concentrates, because of the presence of other secondary metabolites such as tannin and lignin that could inhibit ruminal BH (Willems et al., 2014; Girard et al., 2016; Alves et al., 2017). However, forage generally contains a low level of total FA, ranging from 1-3% dry matter (Chilliard et al., 2007; Jaturasitha et al., 2016). Thus, other lipid sources are commonly supplemented to increase dietary energy density and improve animal performance (Vazirigohar et al., 2014; Parvar et al., 2017).

2.5.2. Marine products

Feeding marine resources to livestock provides greater opportunities for increasing a vast range of FA products, from n-3 LC-PUFA and also various BH intermediates, although the sustainability (Lenihan-Geels et al., 2013; Kitessa et al., 2014) and cost effectiveness of using marine sources has been questioned (Vlaeminck et al., 2008; Shingfield et al., 2012; Chikwanha et al., 2017). In addition, inclusion of marine sources into ruminant diets may introduce food odours, result in rancidity and add abnormal flavour in lamb meat (Watkins et al., 2013; Scollan et al., 2014). The efficiency of supplementing fish oils in ruminant diets is still controversial (Scollan et al., 2014). Several studies demonstrated that fish oil supplementation into sheep diets substantially increased the levels of n-3 LC-PUFA in lamb meat and ewe milk (Jaworska et al., 2016; Parvar et al., 2017). In contrast, other studies concluded that the increase in the n-3 LC-PUFA content of edible tissues in ruminants fed fish oils are marginal due to extensive BH in the rumen (Shingfield et al., 2012; Bessa et al., 2015). Thus, new and sustainable sources of n-3 LC-PUFA, and their mode of incorporation and application, for supplementation into ruminant diets are required.

Marine algae, an alternative and sustainable source of n-3 LC-PUFA (Kitessa et al., 2014), has been recently included in sheep diets to increase levels of n-3 LC-PUFA in lamb

(Ponnampalam et al., 2016a; Díaz et al., 2017). Overall, marine algae are more effective for the incorporation of n-3 LC-PUFA into muscle and adipose tissues because their component oils have a lower rate of BH compared to fish oils (Howes et al., 2015; Chikwanha et al., 2017). Sinclair (2007) discussed that the structure of the algal cell wall might physically protect the algae n-3 LC-PUFA against access by bacteria and the enzymes involved in BH. However, Urrutia et al. (2016) concluded that use of marine algae have adverse effects on meat quality, with higher lipid oxidation, and reduced odour and flavour rates. However, the high cost for production of algal biomass, extraction and purification is currently limiting the potential of using marine algae on a larger scale in feeding livestock (Lenihan-Geels et al., 2013).

2.5.3. Oilseed and terrestrial plant oils

Oilseed and vegetable oils generally have a greater ratio of UFA to SFA compared with terrestrial animal fats (Woods and Fearon, 2009). Oilseeds and vegetable oils used in ruminant diets provide a rich source of both energy and protein (Petit, 2010), and their lipid composition is generally more than 20% PUFA (Dubois et al., 2007). As they are derived from terrestrial plants, oilseeds and vegetable oils are rich in medium-chain FA, and do not contain n-3 LC-PUFA (Dubois et al., 2007; Jaturasitha et al., 2016). The FA composition of popular vegetable oils are shown in Table 2.3. A number of vegetable oils are rich in linoleic acid, including those from safflower, walnut, sunflower, and soybean (Dubois et al., 2007; Shingfield et al., 2013), while flaxseed, camelina and canola oils are abundant in ALA (Salem and Eggersdorfer, 2015; Baker et al., 2016).

Table 2.3. The fatty acid composition (g/100g total fatty acid) of popular vegetable oils (adapted from Dubois et al. (2007))

Fatty acid[1]	Sun-flower	Safflower	Walnut	Canola	Flax-seed	Rice bran	Soy-bean	Camelina	Peanut
14:0	0.1	ND	0.1	0.1	ND	0.4	0.1	ND	0.1
16:n-7	0.1	0.1	0.4	0.2	0.1	0.2	0.2	ND	0.2
16:0	6.4	6.1	10.4	4.5	6.1	18.2	10.8	5.3	10.4
18:2n-6	64.6	75.8	74.0	21.5	16.8	34.6	52.1	16.0	30.3
18:3n-3	0.5	0.3	10.0	9.9	55.0	1.2	7.8	38.1	0.4
18:1n-9	21.9	13.4	ND	59.1	18.1	41.7	23.9	18.7	47.9
18:0	4.5	2.3	3.9	1.7	3.4	1.9	3.9	3.0	3.0
20:1n-9	0.2	0.2	ND	1.4	ND	ND	0.1	11.6	1.3
20:0	0.3	0.4	0.3	0.3	0.5	0.7	0.3	1.4	1.2
22:1n-9	0.1	ND	ND	0.4	ND	ND	ND	2.5	0.1
22:0	0.8	0.3	0.1	0.3	ND	0.2	0.2	ND	2.3
ΣSFA	12.8	9.1	15.4	7.1	10.0	21.3	15.7	11.1	19.3
ΣMUFA	22.2	13.8	0.6	61.4	18.2	42.8	24.5	33.8	49.9
ΣPUFA	65.0	77.1	84.0	31.5	71.8	35.9	59.8	55.1	30.8
PUFA/SFA	5.1	8.5	5.5	4.4	7.2	1.7	3.8	5.0	1.6
Σn-6 PUFA	65.6	76.5	74.0	21.6	16.8	34.6	52.1	16.0	30.3
Σn-3 PUFA	0.5	0.8	10.0	9.9	55.0	1.2	7.8	38.1	0.4
n-6/n-3	131.2	95.6	7.4	2.2	0.3	28.8	6.7	0.4	75.8

[1]ΣSFA: total saturated fatty acid includes: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ΣMUFA: total monounsaturated fatty acid includes: 14:1, 16:1n-9, 16:1n-7, 16:1n-5, 16:1n-13, 17:1n-9, 18:1n-9, 18:1n-7, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-9, 24:1n-9; ΣPUFA: total polyunsaturated fatty acid includes: 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6,20:5n-3, 20:3n-6, 20:2n-6, 22:5n-6, 22:6n-3, 22:5n-3, 22:4n-6, 24:6n-3, 24:5n-3; Σn-3: total omega-3 PUFA includes 18:3n-3, 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3; Σn-6 PUFA: total omega-6 PUFA includes 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ND: Not detected.

Flax plant (*Linum usitassimum L.*) is a blue flowering annual herb that produces small flat and oval seeds varying from golden yellow to reddish brown colour (Kajla et al., 2015). Flax is an attractive nutrition crop because it contains the high ALA level, high quality proteins and dietary soluble fibre (Bernacchia et al., 2014). Flaxseed, also known as linseed, contains 40 - 45% oil, 20 - 25% protein, 20 - 25% fibre and 1% lignin (Petit, 2010; Ponnampalam et al., 2015). Salem and Eggersdorfer (2015) stated that ALA accounts for approximately 23% of the flaxseed weight. Flaxseed oil contains more than 70% PUFA

with ALA generally contributing around 50% of total FA (Dubois et al., 2007; Baker et al., 2016). Kajla et al. (2015) stated that flaxseed oil is the best terrestrial source of n-3 PUFA.

The canola plant is a bright yellow flowering member of the family *Brassicaceae* that produces small round brown seeds. It includes three main plant species; *Brassica napus*, *Brassica rapa* and *Brassica juncea* (Turchini and Mailer, 2011). Canola seed, also known as rapeseed, contains about 40 - 55% oil, 25 - 38% protein and 15 - 20% fibre (Carré et al., 2016; Wroniak et al., 2016). Although oleic acid is the main FA in canola oil, ALA accounts for around 10% of the total FA (Howes et al., 2015; Baker et al., 2016). Ghazani et al. (2014) stated that canola is one of the most economically important food-oil crops. Production of canola oil is the third greatest globally, after soybean and palm oils. Thus, canola oil is presently one of the most abundant terrestrial sources of n-3 PUFA.

A number of studies have shown that the extent of ruminal BH and the FA profile in meat products may be affected by the type, form and amount of lipid provided to the animals (Ponnampalam et al., 2015; Alves et al., 2017; Chikwanha et al., 2017). Gómez-Cortés et al. (2014) stated that vegetable oils have a greater effect on depressing ruminal PUFA digestion than that of oilseeds. Furthermore, processed oilseeds (rolled, extruded, roasted, ground) are more effective than raw seeds in increasing the concentrations of ALA and total PUFA in lamb (Petit et al., 1997). In contrast, Paim et al. (2014) observed that whole seeds offer some degree of protection against BH as the seed coat limits access of ruminal microorganisms to oil in the seeds. However, intact oilseeds, without some disruptions of the seed coat, may escape digestion completely (Noci et al., 2011). Other studies also reported that the effects of oilseed and vegetable oils on increasing the n-3 LC-PUFA content in ruminants are minor, unless they are protected against BH (Chikwanha et al., 2017; Meignan et al., 2017). Lastly, Kitessa et al. (2009) concluded that the FA profiles

were influenced by duration of oil supplementation as they observed that lambs fed ALA rich diets for a longer period contained more ALA and n-3 LC-PUFA in their meat.

2.6. Effects of plant-derived ALA sources on lambs

In spite of the extensive BH of UFA by ruminal microbes, dietary lipid supplementation is presently the most effective way to manipulate the FA profile of ruminant products (Alvarenga et al., 2015; Jaturasitha et al., 2016; Chikwanha et al., 2017). A range of strategies have been employed to improve lamb growth performance, carcass characteristics (Francisco et al., 2015), plasma metabolites (Parvar et al., 2017) and meat quality, especially the n-3 LC-PUFA profile in edible tissues (Realini et al., 2017). These studies have all used supplementation of plant-derived ALA sources and have culminated in varied degrees of success. The following section will focus on the effect of plant-derived dietary n-3 PUFA on lamb growth and health indexes, tissue FA profile and meat sensory quality.

2.6.1. Animal performance and carcass traits

It is widely accepted that feeding regime can influence animal growth rate and weight gain. When a basal diet (annual ryegrass hay/clover hay) was supplemented with flaxseed (10.7%, DM basis), Burnett et al. (2017) and Ponnampalam et al. (2015) observed that lambs fed the flaxseed supplement had similar dry matter intake (DMI), but greater body weight and carcass yield compared to lambs fed the basal diet alone. Burnett et al. (2012) also concluded that lambs receiving flaxseed, either as whole seed or meal (10%, DM basis) while grazing annual pasture, have greater growth performance than lambs grazing annual pasture alone. These differences would be expected because when the metabolisable energy

(ME) requirements for a growing lamb are not met, flaxseed serves as an energy supplementation source to improve lamb growth response (Burnett et al., 2017).

In isoenergetic and isonitrogenous feeding experiments, lamb growth and carcass parameters were not affected by including up to 10% extruded flaxseed (Urrutia et al., 2015; Urrutia et al., 2016) and 10% extruded canola seed (Berthelot et al., 2010) in the diets. Similar trends in DMI, animal performance and carcass measurements between dietary treatments were also observed when oil seed was included in the diets of lambs at a rate of between 2% and 5% (Meale et al., 2015; Parvar et al., 2017). In addition, Realini et al. (2017) observed similarities in cold carcass weight and fat depth when extruded flaxseed (9%, DM basis) was included in lamb basal diet. Several studies agreed that dietary fat levels at or below 6% dry matter (DM) will not result in any detrimental impact on lamb DMI, growth and carcass traits (Jerónimo et al., 2010; Dávila-Ramírez et al., 2017).

In contrast, high levels of lipid inclusion (> 6%, DM basis) in ruminant diets can result in several negative effects on growth performance. Feeding lipid to ruminants could increase energy density of diets without increasing high-starch concentrate intake or reducing fibre intake, which are both negatively related to rumen function (Scollan et al., 2014; Meignan et al., 2017). Likewise, high-fat diets tend to reduce DMI (Francisco et al., 2015; Parvar et al., 2017) because of the potential decrease in feed palatability (Annett et al., 2011), fibre digestibility (Bhatt et al., 2011) and digestive nutrient flows (Ikwuegbu and Sutton, 1982). Doreau et al. (2009) pointed out that dietary supplementation with high levels of fat and oil act as a toxin to ruminal microorganisms by considerably decreasing the population of protozoa which contributes up to 50% of the biomass in the rumen; this reduction subsequently plays a major role in the increased degradation of protein and fibre (Newbold et al., 2015). Thus, it is suggested that the total fat in the diet should not exceed 6% DM to

avoid the impairment of rumen function, digestibility and DMI (Francisco et al., 2015; Meignan et al., 2017).

2.6.2. Omega-3 PUFA profiles of edible tissues

More recently, emphasis has been placed on increasing the level of n-3 PUFA in lamb editable tissues by feeding canola seed, flaxseed and their oils as sustainable and cost-effective sources (Kitessa et al., 2014). Supplementation with such sources can increase the concentration of ALA in tissue with an associated desirable decrease in the ratio of omega-6 to omega-3 PUFA (n-6/n-3) (Scollan et al., 2014). As shown in Table 2.4, various studies have indicated that inclusion of ALA rich sources in lamb diets generally also increases the level of n-3 LC-PUFA in lamb meat.

There is a pronounced tendency for the level of ALA in intramuscular fat (IMF) to be considerably increased due to ALA supplementation (Table 2.4). Additionally, increases in meat ALA level have been attributed to greater amounts of ALA intake (Jerónimo et al., 2010). Doreau and Ferlay (1994) stated that if large amounts of FA are available in the rumen, it is possible for significant direct uptake of dietary FA to occur. Several *in vitro* studies also confirmed that the extent of BH of n-3 PUFA in ruminants is limited by the high amount of these FA available to rumen microbes, even without rumen protection technologies (Ramos-Morales et al., 2016). Consequently, a part of dietary ALA could escape microbial degradation in the rumen and be directly absorbed in the small intestine and then incorporated into meat and other tissues through the circulatory system (Urrutia et al., 2015; Parvar et al., 2017).

Some studies have reported that ALA supplementation increases n-3 LC-PUFA levels in meat. Asadollahi et al. (2017) demonstrated that the inclusion of 7% roasted canola seed to

Arabian lamb fattening diets significantly enhanced n-3 LC-PUFA content in *Longissimus* muscle. This may have occurred as a result of further biosynthesis by desaturation and elongation of ALA to n-3 LC-PUFA by rumen microbes, as microbial-derived n-3 LC-PUFA may account for up to 30% of n-3 LC-PUFA content in the intestine digesta (Sinclair, 2007). These FA are also absorbed directly in the intestine and then stored in the tissues. However, other studies only observed a substantial increase in EPA in meat when 9% extruded flaxseed (Realini et al., 2017) or a blend of 6% flaxseed oil and sunflower oil (2:1, v/v) were added to lamb finishing diets (Jerónimo et al., 2010); DPA and DHA were not altered. Furthermore, Urrutia et al. (2015) and Parvar et al. (2017) reported no significant difference in n-3 LC-PUFA levels when feeding lamb with ALA rich sources. These findings show that limited conversion from ALA to n-3 LC-PUFA occurs in some cases for ruminants.

In contrast, several studies have found no differences in n-3 PUFA profiles when lambs were supplemented with an ALA rich oil source in finishing diets. Radunz et al. (2009) supplemented a 3% blend of soybean oil and flaxseed oil (2:1, v/v) into lamb finishing diets and observed only modest changes in ALA and overall n-3 LC-PUFA composition.

Table 2.4. Effect of supplementing ALA rich oil sources to lamb on the n-3 PUFA profile of *Longissimus* muscle and the n-6/n-3 ratio[¥]

ALA rich source	unit	ALA	EPA	DPA	DHA	n-6/n-3	Reference
Control[1]	g/100 g FA	0.45	0.48	0.29	0.32	5.8	Parvar et al. (2017)
3% canola oil	g/100 g FA	1.09	0.68	0.44	0.44	4.1	Parvar et al. (2017)
Basal diet[2]	mg/100 g meat	18.0	2.6	5.3	1.2	6.2	Realini et al. (2017)
9% extruded flaxseed	mg/100 g meat	32.0	4.0	6.3	1.4	3.7	Realini et al. (2017)
Control[3]	mg/100 g meat	5.5	2.5	7.6	1.7	4.0	Asadollahi et al. (2017)
7% roasted canola seed	mg/100 g meat	14.7	5.4	15.1	4.3	4.8	Asadollahi et al. (2017)
Control[4]	g/100 g FAME	0.40	0.19	0.23	0.05	10.5	Urrutia et al. (2016)
5% extruded flaxseed and 3.89% marine algae	g/100 g FAME	0.89	1.01	0.32	0.99	4.4	Urrutia et al. (2016)
10% extruded flaxseed	g/100 g FAME	1.84	0.74	0.31	0.08	3.8	Urrutia et al. (2016)
Control[5]	g/100 g FAME	0.47	0.11	0.14	0.05	13.5	Urrutia et al. (2015)
5% extruded flaxseed	g/100 g FAME	0.92	0.12	0.13	0.02	6.5	Urrutia et al. (2015)
10% extruded flaxseed	g/100 g FAME	1.11	0.15	0.11	0.03	5.4	Urrutia et al. (2015)
Control[6]	mg/100 g meat	15.1	NA	NA	NA	3.4	Francisco et al. (2015)
4% flaxseed oil and soybean oil (2:1, v/v)	mg/100 g meat	36.5	NA	NA	NA	2.1	Francisco et al. (2015)
Ryegrass/clover hay	mg/100 g meat	34.3	17.6	13.9	7.6	NA	Ponnampalam et al. (2015)
10.7% flaxseed	mg/100 g meat	59.5	18.1	11.2	6.7	NA	Ponnampalam et al. (2015)
Control[7]	mg/100 g meat	13.7	10.6	13.9	5.7	4.4	Andrés et al. (2014)
8.5% ground flaxseed	mg/100 g meat	24.6	15.8	17.6	7.2	3.1	Andrés et al. (2014)
Control[8]	g/100 g FAME	0.50	0.07	0.20	0.04	5.3	Noci et al. (2011)
6% flaxseed oil	g/100 g FAME	1.74	0.12	0.21	0.03	1.8	Noci et al. (2011)
Basal diet[9]	g/100 g FA	0.70	0.38	0.70	0.24	6.2	Jerónimo et al. (2010)
6% sunflower oil and flaxseed oil (1:2, v/v)	g/100 g FA	2.72	0.59	0.73	0.23	2.5	Jerónimo et al. (2010)
6% sunflower oil	g/100 g FA	0.93	0.19	0.46	0.14	7.04	Jerónimo et al. (2009)
6% sunflower oil and flaxseed oil (2:1, v/v)	g/100 g FA	1.57	0.29	0.54	0.15	3.78	Jerónimo et al. (2009)
6% sunflower oil and flaxseed oil (1:2, v/v)	g/100 g FA	2.62	0.51	0.62	0.19	2.26	Jerónimo et al. (2009)
6% flaxseed oil	g/100 g FA	3.05	0.50	0.54	0.20	1.60	Jerónimo et al. (2009)

[¥] ALA: Alpha-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; FA: fatty acid; FAME: fatty acid methyl esters; NA: data not available. [1]The control diet was a total mixed ration without oil. [2]The basal diet was based on lucerne hay or corn. [3]The control diet was mainly composed of milled barley, lucerne hay, soybean meal and canola meal. [4,5]The control diet was mainly composed of barley and soybean meal. [6]The control diet was composed of lucerne hay and wheat, soybean meal. [7]The control diet was a total mixed ration with palm oil. [8]The control diet was based on Megalac (palm-oil based high in 16:0). [9]The basal diet was composed of lucerne hay and manioc.

Likewise, Berthelot et al. (2012) concluded that n-3 PUFA proportions in lamb meat were not affected when 6% extruded flaxseed was included in the animals diet. Some explanations for this limited success in the incorporation of ALA have been proposed: (1) the extensive BH of the dietary ALA, ranging between 85 and 95% (Glasser et al., 2008; Alves et al., 2017); (2) low endogenous conversion of ALA to n-3 LC-PUFA (Scollan et al., 2001); and (3) the limited capacity of muscle to incorporate and store the n-3 LC-PUFA (Wood et al., 2008; Bessa et al., 2015). However, these studies still confirmed that increasing the dietary n-3 PUFA intake in ruminants is the base of any strategy to enrich red meat in n-3 PUFA levels.

In lamb muscle, DPA tends to be present at a greater relative level and absolute content than EPA and DHA (Table 2.4). The roles of DPA in human health have been largely ignored because it is generally considered as an intermediary between EPA and DHA (Kaur et al., 2016). As previously discussed, the potential health benefits of DPA are now emerging, although it is not listed as a beneficial source of n-3 by many health organisations (Byelashov et al., 2015). Vahmani et al. (2015) stated that the exclusion of DPA from total n-3 LC-PUFA intake results in reducing the total amount of their actual consumption. Moreover, Clayton (2014) stated that inclusion of DPA in n-3 LC-PUFA intake would boost the total n-3 LC-PUFA content of lamb to higher values. Thus, several studies have suggested that DPA should be included in measurement of the total n-3 LC-PUFA intake (Howe et al., 2006; Mozaffarian and Wu, 2012).

The ratio of n-6/n-3 PUFA is an important indicator that is used to evaluate the nutritional value of meat for human consumption, because an increasing ratio is known to positively correlate with the higher risk of CVD (Russo, 2009). A desirable dietary recommendation for the n-6/n-3 PUFA ratio is less than 5/1 and individuals should be encouraged to

consume more n-3 rich foods if their diets has n-6/n3 ratio in excess of 10/1 (WHO/FAO, 1994). It is evident from Table 2.4 that ALA rich supplementation resulted in a significant decrease in the n-6/n-3 ratio with the exception of the study of Asadollahi et al. (2017). Furthermore, the n-6/n-3 ratio of lamb feeding on ALA rich sources tends to achieve the recommended value. The addition of ALA rich seeds or oils to ruminant diets would increase the ALA and potentially the n-3 PUFA levels in the meat products and thereby contribute to a decrease in the n-6/n-3 ratio (Noci et al., 2011).

A large number of studies have been undertaken to investigate the impacts of oilseed and/or terrestrial plant oils on lamb n-3 PUFA profiles, with most studies focusing on the effects on *Longissimus* muscle or intramuscular fat (Table 2.4). Several studies have determined the changes in FA profiles of subcutaneous, perirenal, and caudal fat (Berthelot et al., 2010; Meale et al., 2015), while other studies have investigated the alterations in the composition of ewe milk (Berthelot et al., 2012; Nudda et al., 2014) and edible non-carcass components. The success rate of increasing n-3 PUFA content in edible tissues is controversial. Thus, further studies are required to better define the optimum level of n-3 PUFA supplementation, and the duration of the supplementation, in addition to other factors in order to increase n-3 PUFA content in edible tissues, and limit the risk of adverse effects on animal performance.

2.6.3. Lamb meat eating quality

In addition to nutritive value, sensory characteristics of meat are other key factors which influences consumers (Pethick et al., 2006). Meat palatability can be described by the level of tenderness, juiciness, flavour and overall liking of lamb meat (Pannier et al., 2014a). The nutritional characteristics of diets significantly influence sensory quality of meat (Girard et al., 2016; Jaworska et al., 2016).

More recently, research has focused on the effects of oilseed and vegetable oils on lamb sensory properties. Francisco et al. (2015) included 4% flaxseed oil and soybean oil (2:1, v/v) in lamb diets, and did not find any considerable effect on meat sensory characteristics compared to lambs fed the control diet without oil. A similar result was observed by Urrutia et al. (2016) who included 10% extruded flaxseed in lamb diets. The sensory properties of the *Longissimus* muscles were not affected by the inclusion of 6% vegetable oil in lamb (Jerónimo et al., 2012) and goat diets (Dávila-Ramírez et al., 2013; Dávila-Ramírez et al., 2017). Overall, these studies in combination reveal that feeding small ruminants with dietary plant lipid at or below 6% DM will not result in a deterioration in meat eating quality.

In contrast, Francisco et al. (2015) demonstrated that the supplementation of 8% flaxseed oil and soybean oil (2:1, v/v) in lamb diets had detrimental effects on juiciness, flavour and overall liking rates. A similar finding was also observed by Abuelfatah et al. (2016) who fed goats with 20% whole flaxseed in their diets. They concluded that supplementing ruminant diets with oilseed and/or vegetable oils can increase meat PUFA, although also increase susceptibility to oxidative degradation which may hasten a deterioration in meat quality. In general, the oxidation of PUFA during retail display, processing, and cooking is responsible for alterations in sensory properties, especially meat flavour and overall liking (Nute et al., 2007; Kouba and Mourot, 2011; Jaworska et al., 2016). However, individual FA have different impacts on meat eating quality. Increasing the amounts of the n-3 LC-PUFA, such as EPA and DHA, in lamb meat are linked to greater scores of rancid odour and fish flavour, and consequently less overall liking (Nute et al., 2007; Urrutia et al., 2016). Conversely, increasing ALA content in lamb meat led to increasing flavour and

overall liking (Sanudo et al., 2000; Nute et al., 2007). A similar finding was also observed in meat from goats (Abuelfatah et al., 2016).

A number of studies have demonstrated that IMF plays an important role in sensory properties of meat (Sanudo et al., 2000; Watkins et al., 2013). Asadollahi et al. (2017) found that increased IMF content in the *Longissimus* muscle positively correlated with a significant increase in meat sensory characteristics. High IMF level is directly associated with enhanced juiciness in cooked meat (Wood et al., 2008). Abuelfatah et al. (2016) stressed that juiciness is more influenced by IMF content than individual FA. Pannier et al. (2014a) also concluded that IMF explained the largest amount of variation in juiciness and flavour. Therefore, finishing lambs would have to obtain high IMF levels to reach optimal eating quality. It has been suggested that lambs need to reach 4% IMF in their meat to achieve consumer satisfaction for palatability (Pannier et al., 2014b).

2.6.4. Plasma metabolite responses

Plasma metabolites provide important indicators of the health and nutritional status of animals (Filipović et al., 2011; Kholif et al., 2016). Globulin, albumin and urea are all directly related to protein metabolism and their concentrations depend on the type and amount of dietary protein intake (Malau-Aduli and Holman, 2015). Kumari et al. (2016) reported that the concentrations of non-esterified fatty acids (NEFA) and beta hydroxyl butyrate (BHB) circulating in the blood reflect energy status. Cholesterol is responsible for several vital biochemical processes and is the precursor for the synthesis of endogenous steroid hormones (El-Hamid et al., 2016). Plasma glucose plays an important role in gluconeogenesis pathways sourcing carbon from complex carbohydrates, lipids, and protein (Malau-Aduli and Holman, 2015). Hence, quantifying main plasma metabolite concentrations has been applied to measure the response of lambs to alternative diets and

n-3 PUFA enriched supplements (Ponnampalam et al., 2015; Parvar et al., 2017). A number of non-isoenergetic and non-isonitrogenous studies have reported increased plasma concentrations of metabolites in ruminants with dietary supplementation of vegetable oil. The inclusion of 10.7% flaxseed in lamb diets resulted in an increase in cholesterol and triglyceride concentrations (Ponnampalam et al., 2015). A similar finding was observed by Ye et al. (2009) who supplemented Holstein dairy cow diets with 2% flaxseed oil. Kholif et al. (2016) also concluded that 2.3% flaxseed oil supplementation increased the plasma glucose concentration of lactating goats. A possible explanation for these differences is that flaxseed addition increases the energy density of the diet compared to oil supplemented diets, causing increases in energy intake and changes in fat mobilization and deposition in animal tissues (Ye et al., 2009). Furthermore, the increased cholesterol concentrations could be due to a decrease in the circulation of low-density lipoprotein cholesterol in the blood via flaxseed supplementation (Pan et al., 2009). Increased organic matter digestibility, total volatile fatty acids, and propionate concentrations occurring with oil supplementation are likely the main reasons for the elevated plasma glucose observed in oil-supplemented diets compared to the control (Kholif et al., 2016).

Parvar et al. (2017) supplemented lamb diet with 3% canola oil and reported that plasma concentrations of cholesterol, glucose and urea were not significantly affected by the supplementation. Borhani et al. (2016) concluded that the inclusion of 15% whole canola seed in finishing lamb diets did not result in any great variation in blood metabolite profiles. Similarly, vegetable oil contents up to 5% of total diets did not alter the metabolite concentrations of goats (Roy et al., 2013) and Holstein-Friesian cows (Otto et al., 2014). The authors explained that the absence of significant differences in the metabolite concentrations could be related to the similarity of ME and crude protein components

between the diets. Oil levels at or below 6% DMI avoid the possibility of negative effects on DMI and nutrient digestibility (Bhatt et al., 2016). As a consequence, the inclusion of dietary lipids derived from ALA rich sources at or below 6% (DM basis) in isoenergetic and isonitrogenous diets favours healthy metabolite concentrations in the ruminant blood.

2.6.5. Wool quality

Apart from meat, wool also contributes a significant proportion to economic returns in dual-purpose enterprises (Holman and Malau-Aduli, 2014). Wool quality characteristics are intrinsically linked to market demand and wool price (Mortimer et al., 2010; Cottle et al., 2013; Nolan et al., 2014). Wool quality has been routinely and objectively assessed by purchasers to determine trade prices. Producers also assess the wool traits to quantify management choices that influence wool value (Mortimer et al., 2010; McGregor et al., 2016). The wool quality traits will be briefly described.

Mean fibre diameter (FD), measured in microns (μm), displays the average width of a single cross section of wool fibre (Flanders and Gillespie, 2015; Scobie et al., 2015). It is widely acknowledged as the most important wool property when assessing wool quality and value (Rowe, 2010; Nolan et al., 2014), contributing approximately 75% of the total price of raw wool (Mortimer et al., 2010). Fibre standard deviation (FSD) and the coefficient of variation (CV) are used to characterise FD variation within a normal bell-shaped distribution (Botha and Hunter, 2010; Holman and Malau-Aduli, 2012).

Clean fleece yield (CFY) refers to the percentage of wool content after removing grease, epidermal particles, vegetable matter and soil (Rogers and Schlink, 2010; Scobie et al., 2015). CFY is directly responsible for determining the commercial value of wool (Mortimer et al., 2010) because higher prices are offered for wools with greater CFY

(Rogers and Schlink, 2010; Holman and Malau-Aduli, 2012). Comfort factor (CF) is defined as the percentage of wool fibers with a diameter below 30 μm (Malau-Aduli and Deng Akuoch, 2010). CF is particularly beneficial in developing market demand for luxurious wool apparel (Rowe, 2010; Holman and Malau-Aduli, 2012). Fibre curvature (FC) describes crimp frequency as the number of crimps per unit of length (McGregor and Naebe, 2016). Low FC associates with softness of handle or low resistance to compression of both raw and scoured wool (McGregor, 2014). Spinning fineness (SF) is a measure of the performance of the fibre when spun into yarn that considers both FD and CV of the wool sample (Rafat et al., 2007). Malau-Aduli et al. (2012a) stated that SF permits accurate comparison and estimation of wool processing speed, cost, and yarn evenness, and low SF wool is typically more desirable and financially rewarded. It is therefore a sensible alternative to FD, for use as a selection attribute for wool sheep (Butler and Dolling, 1992).

Wool is not a uniform biological product because its physical quality characteristics vary depending on sheep genetics, environment and management strategies (Khan et al., 2012; Nolan et al., 2014). Wool production and quality are also influenced by the age and sex of animals, reproduction in the ewe, and the position of staples (Khan et al., 2012; Scobie et al., 2015). There are definite differences between breeds of sheep in the capacity to grow wool and in various fleece quality characteristics (Thompson et al., 2011). Likewise, within a breed, there is considerable variation in the rate of wool growth between strains and individual sheep (Khan et al., 2012). However, various studies have confirmed that variations in nutrition can exert considerable influence on fibre production and the quality of wool (Thompson et al., 2011; Khan et al., 2012; McGregor et al., 2016). The quality and amount of dietary protein are important for wool synthesis and growth, as wool is composed of almost entirely protein with very high levels of cysteine and serine compared

with other body tissues (Plowman, 2003; Khan et al., 2012). Growth of wool requires more protein relative to energy, and draws amino acids, particularly methionine and cysteine (Sahoo and Soren, 2011; Holman and Malau-Aduli, 2014). Thus, nutrition research on wool production mainly focuses on the use of protein-rich supplements. Investigations on wool quality responses to lipid supplementation have received little attention in the current literature.

More recently, several studies have examined the effects of lipid supplementation on wool properties. Malau-Aduli et al. (2014) fed up to 5% (DM basic) degummed canola oil to Australian finishing lambs, but did not find any considerable effect on wool quality. Likewise, Meale et al. (2014) reported that neither wool production nor quality was affected by dietary inclusion of up to 3% (DM basic) marine microalgae to growing lambs. A similar result was observed by Meale et al. (2015) who included 2% of various vegetable oils in finishing lamb diets. Interestingly, the inclusion of lipid in these studies did not alter the crude protein and metabolisable energy contents, and dry matter intake (DMI) in lamb diets. These could partly explain the fact that wool quality remained unchanged during lipid supplementation. Wool growth and quality is substantially influenced by DMI (Hynd and Masters, 2002; Rangel and Gardiner, 2009). Thus, these studies in combination reveal that feeding lamb with dietary lipid in isoenergetic and isonitrogenous diets will not result in differences in wool attributes.

2.7. Summary

The Australian sheep industry has experienced a substantial change over the last two decades. Wool production has drastically reduced by more than a half, both in total yield and on-farm economic value. Conversely, sheep meat has taken precedence over wool and become the key driver of the sheep industry. Sheep production has shifted to dual-purpose prime lamb systems from predominantly wool-only farming. Genetics improvement for economically important characteristics, such as growth performance, muscle development, carcass, sensory quality and wool fibre diameter, has received considerable attention from researchers, breeders and producers. MERINOSELECT and LAMBPLAN have been employed to produce animals that have (1) high productivity, excellent reproductive performance and desired product quality, (2) resistance to diseases and hard environmental conditions, and (3) ability to meet market demand. However, most Australian lambs are raised in extensive grazing systems. In these systems, lamb productivity depends on environmental factors which influence feed availability and pasture growth. Concentrates need to be used to supplement sheep to satisfy their nutritional requirements when pasture availability is limited. To optimise growth rate, feed conversion efficiency and reduce the number of days on feed, lamb producers have frequently used feedlotting during the finishing period.

Omega-3 PUFA are widely accepted as an essential component of modern nutrition. They contribute a major constitute in brain and retinal tissues and have protective effects against CVD, cancer and inflammatory diseases. Although daily n-3 LC-PUFA intakes have been recommended by various health organisations worldwide, the median amount of n-3 LC-PUFA consumption is generally lacking in Western diets. The richest sources of dietary n-3 LC-PUFA are generally seafood, but it not a regular part of traditional Western diets. This creates opportunities to improve for n-3 LC-PUFA content in other human foods such

as lamb meat. Recent evidence suggests that n-3 LC-PUFA content in lamb would be improved through dietary lipid supplementation.

The complexity of ruminant metabolism, determined mainly by processes of lipolysis and BH of dietary lipid in the rumen, will continue to challenge the efforts of producers to modulate meat quality including its n-3 LC-PUFA profiles. However, it is widely accepted that dietary lipid, which is mainly derived from grasses, marine products, oilseed or their oils, is the major factor manipulating the FA profile of ruminant products. Feeding marine resources seems to result in effective responses in the increased content of n-3 PUFA in the animals. Nevertheless, they might also have adverse effects on meat eating quality, especially odour and flavour. The unsustainability and high cost are also current limitations to the utilisation of marine products. Thus, flaxseed, canola seed and their oils rich in ALA are considered as alternative and sustainable sources of n-3 PUFA supplementation into lamb diets to increase content of the health-benefitting n-3 LC-PUFA.

The responses of lamb performance and meat quality to ALA rich supplementation have been inconsistent to date. In low energy density diets, supplementation can improve lamb growth, carcass traits and eating quality. Conversely, supplementing with lipid derived from ALA rich sources at or below 6% DM to isoenergetic and isonitrogenous diets seems unlikely to influence lamb DMI, growth, carcass and sensory properties and plasma metabolite concentrations and wool quality. The increase in n-3 LC-PUFA content due to ALA rich supplementation that have been reported are rather inconsistent. Several studies concluded that ALA rich supplementation led to an increase in n-3 LC-PUFA content, while others found no differences in these FA when lamb were supplemented with ALA rich sources in finishing diets.

Therefore, it is necessary to perform further studies that simultaneously investigate nutritional and genetic effects on lamb performance and products (edible tissues and wool) in an on-farm intensive management system.

Chapter 3: Feed intake, growth and wool quality responses of genetically divergent Australian lambs to canola oil or flaxseed oil supplementation

3.1. Abstract

The effects of graded levels of canola and flaxseed oil supplementation, breed, gender and their second-order interactions on lamb dry matter intake (DMI), growth and wool quality traits were evaluated. Sixty dual-purpose prime lambs, including purebred Merino and crossbred lambs, were allocated to one of five treatments of lucerne hay basal diet supplemented with isocaloric and isonitrogenous wheat-based pellets. Treatments were: without oil inclusion (Control); 2.5% canola oil; 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil, with lamb groups balanced by breed and gender. Each lamb was daily supplemented with one kg of pellets and had free access to lucerne hay and water throughout the 7-week feeding trial, after a 3-week adaptation. Individual animal basal and supplementary pellet feed intakes were recorded daily, while body conformation traits, body condition scores and liveweights were measured on days 0, 21, 35 and 49. The lambs were shorn before commencing the feeding trial and mid-side wool samples were collected from each lamb at the end of the experiment. Oil supplementation had no detrimental effect on lamb DMI, growth performance and wool quality traits ($P > 0.05$). Lamb breed elicited significant differences in DMI, chest girth gain and wool quality ($P < 0.05$). Gender significantly affected wither height gain and fibre diameter. There were significant interactions between oil supplementation and lamb breed on feed efficiency and growth traits. Combinations of CxM lambs fed 5% canola oil and WxC lambs fed 5% canola oil are effective and strategic management tools for enhancing feed efficiency and growth without negative effects on wool quality in dual-purpose lamb production.

3.2. Introduction

The increased incidences of central nervous system disorders, cardiovascular diseases and cancers have been associated with high consumption of red meat (Ekmekcioglu et al., 2017; Wolk, 2017), with associated high levels of saturated (SFA) and low omega-3 polyunsaturated (n-3 PUFA) fatty acid contents (Bessa et al., 2015). Previous studies had demonstrated that n-3 PUFA content in meat products can be manipulated by supplementing ruminants with feeds enriched with n-3 PUFA dietary sources (Ponnampalam et al., 2015; Jaworska et al., 2016; Asadollahi et al., 2017) that include fish, algae, oilseeds and their oils (Woods and Fearon, 2009; Nichols et al., 2010). The practical inclusion of marine products in ruminant supplements is unsustainable due to prohibitively high cost and scarcity (Lenihan-Geels et al., 2013; Kitessa et al., 2014), as well as concerns regarding detrimental effects on sensory eating quality (Scollan et al., 2014; Urrutia et al., 2016). Thus, oilseeds and their oils are considered as more practical alternative and sustainable sources of n-3 PUFA. Canola and flaxseed oils contain an abundance of α-linoleic acid (ALA, 18:3n-3) (Gillingham et al., 2011; Ding et al., 2017) and have been of recent interest in a number of feeding trials aiming to increase n-3 PUFA levels in lamb (Urrutia et al., 2015; Francisco et al., 2016; Flakemore et al., 2017). However, these investigations mainly focused on variations in meat fatty acid profiles of lambs fed vegetable oils. Research on animal feed intake, growth and wool quality responses of lambs to dietary oils rich in ALA have received little attention and even more limited on-farm research has been conducted on the optimal supplementary level and duration of feeding these oils in lamb diets.

Due to decreased wool prices in the past two decades, the Australian sheep industry has adopted dual-purpose sheep systems with both wool and meat production goals (Rowe,

2010; Malau-Aduli and Akuoch, 2012). Genetic management of animals for enhancing both growth performance and wool quality in dual-purpose sheep systems through crossbreeding strategies provides a cumulative and long-term alternative approach to nutritional manipulation (Malau-Aduli et al., 2014). The effects of sheep breed and gender on growth responses and wool traits have been reported (Holman et al., 2014a; Holman et al., 2014b; Gardner et al., 2015; Walkom and Brown, 2017). However, these studies were conducted under grazing conditions with seasonal variability in pasture supply. Studies simultaneously comparing growth and wool attributes between sheep breeds in intensive lamb production systems during finishing periods remain scarce. Previous studies had investigated lamb growth rates and slaughter weights in response to energy-rich supplements in research stations without investigating associations with wool quality traits. In contrast, the current study is unique in its integrated approach in a typical 'real world' on-farm, dual-purpose and intensive crossbred lamb finishing system. Its uniqueness is further justified by an attempt to fill the currently existing knowledge gap on the appropriate supplementary levels and associated impacts of flaxseed and canola oils on feed intake, growth and wool quality of Australian prime lambs from different genetic backgrounds. This present study did not only focus on the variations in growth, body conformation and wool quality traits over the duration of the feeding trial, but also investigated second-order interactions between oil supplementation, sheep breed and gender combinations in an attempt to provide sheep farmers with a variety of choices for targeting optimal slaughter weights and wool quality traits. Therefore, the objectives of this study were to investigate feed intake, growth and wool quality responses of ewe and wether Australian dual-purpose prime lambs from three different breeds supplemented with graded levels of either canola oil or flaxseed oil based pellets in an on-farm intensive

finishing management system. It also aimed to estimate residual phenotypic correlations within and between wool quality traits and lamb performance under the same management system.

3.3. Materials and methods

This on-farm research was conducted under the auspices of the Tasmanian Institute of Agriculture Cressy, Tasmania, Australia from June to August, 2014. The experimental design and protocols were approved by the University of Tasmania Animal Ethics Committee (Permit No. A13839) and followed the 2013 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

3.3.1. Animals and experimental design

Single-born prime lambs weighing between 4.5 and 5.5 kg were subjected to on-farm best practice operations of marking, vaccination, castration and tail-docking at about 12-14 weeks of age when they were weaned. Sixty weaner ewe (n = 30) and wether (n = 30) lambs, 6-7 months old with an average liveweight (LWT) of 33.4 ± 0.7 kg and body condition score (BCS) of 2.7 ± 0.3 were utilised in this study. The completely randomised experimental design comprised 20 purebred Merinos (MxM), 20 Corriedale × Merino (CxM) and 20 White Suffolk × Corriedale (WxC) first-cross lambs with equal number of ewe and wether lambs represented in each breed. Each lamb was supplemented daily with one kg of isocaloric and isonitrogenous wheat-based pellets and randomly allocated to one of five treatments of 12 lambs per group, balanced by breed and gender. The treatments were: no oil inclusion (Control); 2.5% canola oil; 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil on dry matter basis. Lambs were fed for 7 weeks after a three-week adaptation

period and had unlimited access to lucerne hay and clean water. They were offered fresh feed at 09.00 hours after residual feed left-over had been weighed and removed.

3.3.2. Feed intake and growth measurements

The amount of offered pellet and lucerne hay and residual left-over feed were separately weighed daily to calculate feed intake. Representative feed samples were collected on days 0, 25 and 49 of the experimental period and stored at -20°C for subsequent analyses.

Lambs were weighed and their body conformations were measured on days 0, 21, 35 and 49 of the experiment before receiving their daily ration. Liveweights were measured using a calibrated electronic scale (Ruddweigh 3000XT; Gallagher Group, Hamilton, New Zealand). Lamb LWT on initial and final days were obtained to calculate average daily gain (ADG).

The body conformation measurements of chest girth (CG), body length (BL) and wither height (WH) were taken using a measuring tape and as outlined in detail by Holman et al. (2014a). Body condition scores (BCS) were also subjectively determined on a scale ranging from 0 (emaciated) to 5 (obese) by feeling the layer of tissue (fat and muscle) at the short rib region using the thumb and fingers on the ribs as described by McLeod et al. (2010). All measurements were made by the same researcher throughout the trial to minimize variations; lambs were in a relaxed state and restrained, with heads comfortably erect and standing stably upon all four legs.

3.3.3. Wool sampling and analysis

All lambs were shorn a month before the commencement of the trial. At the end of the experiment, wool was clipped from a mid-side patch (10 cm by 10 cm) of each lamb by an experienced shearer using electric shears (Oster-Sunbeam, Boca Raton, FL, USA), as

described by Langlands and Wheeler (1968). Wool quality traits were commercially evaluated at the Australian Wool Testing Authority (AWTA) using Sirolan Laserscan (AWTA Limited, Melbourne, VIC, Australia). Wool quality traits include clean fleece yield (CFY), mean fibre diameter (FD), fibre standard deviation (FSD), coefficient of variation (CV), comfort factor (CF), fibre curvature (FC) and spinning fineness (SF) and these have been described in detail by Holman and Malau-Aduli (2012). In brief, CFY, expressed as a percentage, refers to the fibrous content of wool (Rogers and Schlink, 2010). FD refers to the average width of a single cross section of wool fibre (Flanders and Gillespie, 2015) and it is widely acknowledged as the most important wool property when assessing wool quality and value (Rowe, 2010). FSD is a measurement of fibre diameter variation within a normal distribution, while CV is a refinement of FSD because it is derived from FSD values divided by FD and expressed as a percentage (Holman and Malau-Aduli, 2012). CF is defined as the percentage of wool fibres with diameter below 30 μm (Notter et al., 2007; Malau-Aduli and Deng Akuoch, 2010). FC describes crimp frequency as the number of crimps per unit of length (McGregor and Naebe, 2016). Low FC is associated with softness of handle or low resistance to compression of both raw and scoured wool (McGregor, 2014). SF is a measure of the performance of the fibre when spun into yarn and it takes into account both FD and CV of the wool sample (Notter et al., 2007). Malau-Aduli et al. (2012a) stated that SF permits accurate comparison and estimation of wool processing speed, cost, and yarn evenness, and low SF wool is typically more desirable and financially rewarded. It is therefore a sensible alternative to FD, as a selection attribute for wool sheep (Butler and Dolling, 1992).

3.3.4. Feed chemical analysis

At the end of the experiment, all feed samples were defrosted, pooled by treatments, and ground through a 1-mm screen. Samples were dried in triplicates in a fan-forced oven at 65°C to a constant weight to determine dry matter (DM) content. Total Nitrogen (N) value was quantified using an elemental analyser (PE2400 Series II; Perkin-Elmer Corp, USA) and crude protein (CP) content was estimated by multiplying N by 6.25. Ether extract (EE) was determined using an ANKOM fat/oil extractor (ANKOM^{XT15}; ANKOM Technology, USA). An ANKOM fibre analyser (ANKOM220; ANKOM Technology, USA) was used to measure acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents. The samples were combusted in a furnace at 550°C for 5 hours to quantify ash content. Non-fibrous carbohydrates (NFC) was calculated as NFC = 100 − (CP + NDF + EE + Ash) (Mertens, 2002). A near infrared reflectance spectroscopy method was used to estimate metabolisable energy (Garnsworthy and Unal, 2004). The ingredients and chemical composition of experimental feeds are shown in Table 3.1.

3.3.5. Statistical analysis

Feed efficiency (FE) was computed as gram LWT gain per kilogram of DM feed consumed (Ferreira et al., 2014). Liveweight, body conformation measurements and BCS were transformed into changes (Δ) between the initial and final values for each of the traits over the duration of the feeding trial.

All collected data were analysed using the Statistical Analysis System software version 9.2 (SAS, 2014). General Linear Model (PROC GLM) analyses were used to fit supplementation, sheep breed, gender and their second-order interactions as fixed effects and feed intake, growth performance and wool quality characteristics as dependent variables. The final statistical model used for the analysis was: $Y = \mu + O_i + B_j + G_k + (OB)_{ij} + (OG)_{ik} + (BG)_{ik} + e_{ijk}$ where Y = dependent variable, μ = overall mean, O_i = oil

supplementation, B_j = breed, G_k = gender, brackets represent second-order interactions and e_{ijk} = residual error.

The means were obtained using the LSMEANS option. Significant differences and mean separations were performed using Tukey's probability pairwise comparison tests. Significant effects were declared at $P < 0.05$. Pearson's correlation coefficients between wool quality traits were also estimated using PROC CORR procedure in SAS and significance established using Bonferroni probability pairwise comparison test.

Table 3.1. Experimental pellet ingredients and chemical composition of feed

	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
Ingredients, g/kg						
Wheat	513	537	545	551	465	–
Paddy rice	260	230	210	220	280	–
Lupins	170	151	138	147	148	–
Canola oil (ml/kg)	-	25	50	-	-	–
Flaxseed oil (ml/kg)	-	-	-	25	50	–
Salt	10	10	10	10	10	–
Limestone	21	21	21	21	21	–
Sheep premix[1]	1	1	1	1	1	–
Ammonium sulphate	12.6	12.6	12.6	12.6	12.6	–
Acid buffer	6.2	6.2	6.2	6.2	6.2	–
Sodium bicarbonate	6.2	6.2	6.2	6.2	6.2	–
Chemical composition[2] (%)						
Dry matter, (%)	89.8	90.2	87.9	90.5	89.4	89.6
CP	14.7	14.5	14.4	14.5	14.5	17.4
ADF	9.2	9.3	8.9	9.5	9.0	30.9
NFC	50.5	49.9	47.8	50.5	50.7	27.4
EE	4.5	4.6	4.9	4.7	5.0	1.5
Ash	8.0	7.5	8.2	7.1	6.4	7.2
NDF	23.8	23.5	23.9	23.7	23.3	46.5
ME, MJ/kgDM	10.8	10.9	11.1	10.8	11.0	9.8

[1]Sheep premix (1 g) contained 25,000 IU vitamin A, 2,500 IU vitamin D3, 90 mg vitamin E, 250 mg zinc, 175 mg cobalt, 17 mg iodine, 3 mg manganese and 0.5 mg selenium.

[2]CP: Crude protein; ADF: Acid detergent fibre; NDF: neutral detergent fibre; EE: Ether extract; NFC: Non-fibrous carbohydrates [NFC = 100 − (CP + NDF + EE + ash)]; ME: Metabolisable energy.

3.4. Results

3.4.1. Experimental feed, dry matter intake and average daily gain

The supplemental pellet ingredients and chemical composition of the dietary treatments are presented in Table 3.1. Wheat was the major carrier ingredient in the pellets (465 – 551 g/kg) and the diets were formulated to have similar DM, CP and EE contents between the treatment pellets (P>0.05).

Table 3.2. Average daily gain, dry matter intake and feed efficiency responses of lambs to omega-3 oil supplementation, breed, gender and interactions

Item[1]	Initial LWT (kg)	Final LWT (kg)	ADG (g)	DMI (kg/d)			FE (g/kg)
				Pellet	Lucerne hay	Total	
Treatment							
Control	35.6	44.3	182	0.8	0.7	1.5	119
2.5% canola	37.1	46.2	189	0.8	0.7	1.5	126
5% canola	35.5	45.3	205	0.8	0.7	1.5	134
2.5% flaxseed	36.4	45.2	184	0.9	0.7	1.6	118
5% flaxseed	36.0	45.8	205	0.9	0.7	1.6	132
Breed[2]							
MxM	35.7	44.5	183	0.8[b]	0.7[b]	1.5[b]	122
CxM	36.0	45.6	200	0.8[b]	0.7[b]	1.5[b]	132
WxC	36.7	46.0	195	0.9[a]	0.8[a]	1.7[a]	123
Gender							
Ewes	36.5	45.9	197	0.8	0.7	1.5	130
Wethers	35.8	45.9	188	0.8	0.7	1.5	122
SEM[3]	0.3	0.4	4	0.02	0.02	0.02	2.6
P-values							
Treatment	-	0.52	0.08	0.12	0.33	0.07	0.06
Breed	-	0.20	0.11	0.01	0.01	0.01	0.10
Sex	-	0.13	0.19	0.95	0.12	0.11	0.06
Treatment x Breed	-	0.19	0.03	0.19	0.66	0.97	0.01
Treatment x Sex	-	0.79	0.70	0.21	0.33	0.62	0.28
Sex x Breed	-	0.36	0.37	0.68	0.15	0.66	0.26

[1]LWT: Liveweight; ADG: Average daily gain; DMI: Dry matter intake; FE: Feed efficiency (g of LWT gain/kg of feed)

[2]MxM: purebred Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale

[3]SEM: standard error of the mean.

[a,b]means with different superscripts within a fixed factor significantly differ (P < 0.05).

The ME contents ranging from 10.8 MJ/kg DM to 11.1 MJ/kg DM were also similar between the five oil based supplementary pellets. The CP and ME contents of the basal diet of lucerne hay were 17.4% DM and 9.8 MJ/kg DM respectively.

Daily feed intake was significantly affected ($P < 0.01$, Table 3.2) by lamb breed. First-cross WxC lambs had significantly greater DMI (1.7 kg/d) than MxM and CxM lambs (1.5 kg/d). However, lamb breed did not result in significant differences in final LWT, ADG and FE. The effects of dietary oil inclusion and gender on final LWT, ADG, DMI and FE did not reach the significant threshold ($P > 0.05$).

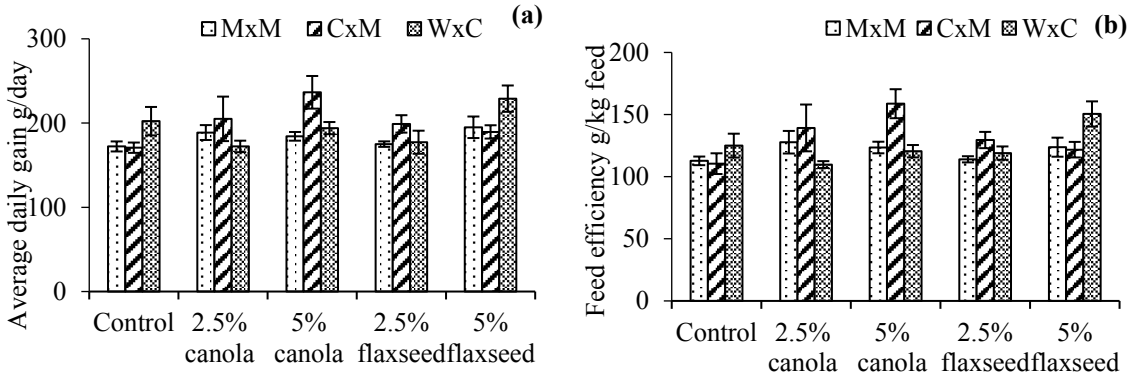

Figure 3.1. Omega-3 oil supplementation and breed interactions on: a) average daily gain and b) feed efficiency of experimental lambs (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale).

Significant effects of oil supplementation and breed interaction on ADG and FE were detected ($P < 0.05$; Table 3.2 and Figure 3.1). First-cross CxM lambs fed pellets containing 5% canola oil and WxC lambs in the 5% flaxseed oil treatment recorded the greatest ADG (236 g and 229 g respectively), and their ADG were significantly greater than those of CxM and MxM lambs in the control treatment, and WxC lambs offered 2.5% canola oil pellets

(Figure 3.1a). Similarly, FE ratios were greatest in CxM lambs fed 5% canola oil pellets (159 g gain /kg feed) and WxC lambs offered pellets containing 5% flaxseed oil (151 g gain /kg feed) (Figure 3.1b).

3.4.2. Changes in liveweight, body conformation and body condition score

The inclusion of oil in pellets resulted in significant differences in CG with the greatest CG gain (136 mm) observed in lambs fed the 5% canola oil treatment ($P < 0.01$; Table 3.3). However, other conformation traits, total LWT gain and body condition score of lambs were unaffected by oil supplementation ($P > 0.05$). Lamb breed and gender were significant sources ($P < 0.01$) of variations in lamb body conformation as the CG gain of WxC lambs (125 mm) was greater than that of other lambs (101 mm), and WH gain was greater in wethers (70 mm) than ewes (55 mm).

Table 3.3 and Figure 3.2 illustrate interactions ($P < 0.05$) between oil supplementation and lamb breed on total LWT and CG gain. It was evident that CxM lambs fed 5% canola oil pellets had greater total LWT gain than MxM and CxM lambs offered the control pellets, and WxC lambs in the 2.5% canola oil treatment (Figure 3.2a). The WxC lambs fed pellets containing canola oil and CxM lambs offered 5% canola oil pellets recorded the greatest CG gain (Figure 3.2b).

Table 3.3. Effects of omega-3 oil supplementation, breed, gender and their interactions on changes (Δ) in liveweight, body conformation measurements and body condition score

Item[1]	ΔLWT (kg)	ΔCG (mm)	ΔBL (mm)	ΔWH (mm)	ΔBCS
Treatment					
Control	8.7	104[b]	74	64	0.4
2.5% canola	9.1	111[b]	58	54	0.6
5% canola	9.8	136[a]	63	58	0.5
2.5% flaxseed	8.8	95[b]	65	63	0.3
5% flaxseed	9.8	98[b]	72	74	0. 7
Breed[2]					
MxM	8.8	101[b]	69	58	0. 5
CxM	9.6	101[b]	70	65	0. 5
WxC	9.4	125[a]	62	65	0.6
Gender					
Ewes	9.0	103	67	55[b]	0.5
Wethers	9.5	115	66	70[a]	0.4
SEM[3]	0.2	5	3	3	0.1
P-values					
Treatment	0.07	0.01	0.31	0.26	0.26
Breed	0.10	0.01	0.39	0.55	0.51
Sex	0.18	0.10	0.85	0.01	0.26
Treatment x Breed	0.03	0.02	0.55	0.18	0.91
Treatment x Sex	0.70	0.17	0.38	0.76	0.16
Sex x Breed	0.36	0.17	0.70	0.85	0.70

[1] LWT: Liveweight; CG: Chest girth; BL: Body length; WH: Withers height; BCS: Body condition score

[2] MxM: purebred Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale.

[3] SEM: standard error of the mean.

[a,b] means with different superscripts within a fixed factor significantly differ ($P < 0.05$).

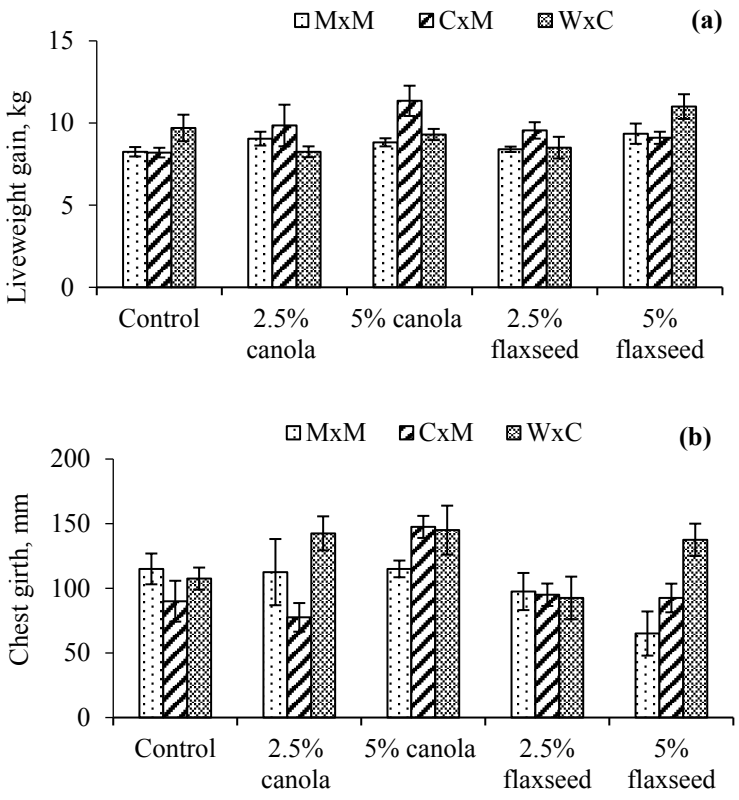

Figure 3.2. Interactions between omega-3 oil supplementation and breed on: a) total liveweight gain and b) chest girth of experimental lambs (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale).

3.4.3. Wool quality attributes

Lamb breed was a significant source of variations in wool quality ($P < 0.01$; Table 3.4). Purebred Merino lambs had greater CFY (76.5%), CF (99.5%), and less FD (17.4 μm), FC (53.5°/mm) and SF (16.4 μm) compared with first-cross lambs studied. Furthermore, variations in fibre diameter (FSD and CV) were less in purebred Merinos than in crossbred lambs. Between two crossbreeds, similarity in wool quality traits were observed, with the exception of FC. Crossbred CxM lambs showed lower FC (70.1°/mm) than WxC lambs (76.3°/mm) ($P < 0.05$).

Table 3.4. Variation in wool quality as influenced by omega-3 oil supplementation, breed, gender and their interactions

Item[1]	CFY (%)	FD (μm)	FSD (μm)	CV (%)	CF (%)	FC (°/mm)	SF (μm)
Treatment							
Control	73.1	22.9	5.0	21.5	83.1	62.8	22.5
2.5% canola	72.7	23.0	4.5	19.1	84.7	67.2	22.1
5% canola	72.4	22.4	4.3	19.0	89.3	66.2	21.5
2.5% flaxseed	75.3	21.8	4.2	19.2	92.1	66.5	20.9
5% flaxseed	72.8	22.1	4.4	19.5	89.3	70.4	21.3
Breed[2]							
MxM	76.5^{a}	17.4^{b}	3.0^{b}	17.2^{b}	99.5^{a}	53.5^{c}	16.4^{b}
CxM	70.8^{b}	25.3^{a}	5.2^{a}	20.7^{a}	79.7^{b}	70.1^{b}	24.6^{a}
WxC	72.5^{b}	24.7^{a}	5.2^{a}	21.0^{a}	83.9^{b}	76.3^{a}	24.0^{a}
Gender							
Ewes	72.9	23.0	4.7^{a}	20.1	85.9	68.3	22.3
Wethers	73.6	21.9	4.2^{b}	19.2	89.6	64.9	21
SEM[3]	0.5	0.6	0.2	0.4	2.0	1.7	0.6
P-values							
Treatment	0.20	0.84	0.26	0.07	0.47	0.07	0.72
Breed	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Sex	0.44	0.15	0.05	0.14	0.30	0.14	0.10
Treatment x Breed	0.58	0.77	0.91	0.51	0.81	0.51	0.85
Treatment x Sex	0.62	0.57	0.72	0.80	0.65	0.80	0.58
Sex x Breed	0.85	0.55	0.15	0.16	0.68	0.16	0.40

[1]CFY: clean fleece yield; FD: mean fibre diameter; FSD: fibre standard deviation; CV: coefficient of variation; CF: comfort factor; FC: fibre curvature; SF: spinning fineness.

[2] MxM: purebred Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale.

[3] SEM: standard error of the mean.

[a,b,c] means with different superscripts within a fixed factor significantly differ ($P < 0.05$).

Fibre standard deviation was influenced by gender, with ewe produced wool having greater FSD than wethers ($P < 0.05$; Table 3.4). However, gender had no significant effects on other wool quality traits. Wool quality traits were unaffected by the inclusion of canola and

flaxseed oils in the pellets, compared with the control group (P > 0.05). No significant second-order interactions on wool quality traits were detected.

3.4.4. Correlations between wool quality traits

There were significant correlations between wool quality traits (Table 3.5). The relationships between CFY and other wool quality traits were moderate ranging from 0.29 to 0.55. Moderate relationships between FC and the other traits were also observed. Moderate to high correlations between FD and other wool quality traits were detected (0.46–0.99) with the strongest relationship between FD and SF. The relationship between CFY and CF were positive, while negative relationships between CFY and the others were observed. As portrayed in Table 3.6, all the correlations between wool quality traits and lamb performance were non-significant (P>0.05).

Table 3.5. Pearson's residual correlation coefficients between wool quality traits

Item[1]	FD	FSD	CV	CF	FC	SF
CFY	-0.43***[2]	-0.41**	-0.29*	0.32*	-0.55***	-0.44***
FD		0.91***	0.53***	-0.91***	0.64***	0.99***
FSD			0.84***	-0.84***	0.60***	0.94***
CV				-0.48***	0.41**	0.62***
CF					-0.44***	-0.91***
FC						0.64***

[1] CFY: clean fleece yield; FD: mean fibre diameter; FSD: fibre standard deviation; CV: coefficient of variation; CF: comfort factor; FC: fibre curvature; SF: spinning fineness.
[2] * P < 0.05; ** P < 0.01; *** P < 0.001.

Table 3.6. Pearson's residual correlations between wool traits and lamb performance

Item[1]	CFY	FD	FSD	CV	CF	FC	SF
ADG	-0.29	0.23	0.17	0.05	-0.16	0.27	0.22
LWG	-0.29	0.23	0.17	0.05	-0.16	0.27	0.22
CG	0.06	0.13	0.14	0.12	-0.11	0.04	0.13
BL	-0.03	-0.13	-0.22	-0.25	0.16	0.00	-0.15
WH	-0.01	0.13	0.16	0.15	-0.06	0.28	0.13
BCS	0.04	0.13	0.14	0.09	-0.15	0.10	0.14

[1]ADG: Average daily gain; LWT: Liveweight gain; CG: Chest girth gain; BL: Body length gain; WH: Withers height gain; BCS: change in body condition score; CFY: clean fleece yield; FD: mean fibre diameter; FSD: fibre standard deviation; CV: coefficient of variation; CF: comfort factor; FC: fibre curvature; SF: spinning fineness.

3.5. Discussion

The results illustrate that supplementation with wheat-based pellets enriched with canola and flaxseed oils had no detrimental effect on DMI. Our findings are consistent with the previous studies of Manso et al. (2009) and Meale et al. (2015) which found no decrease in DMI when lamb diets were supplemented with 2 – 5% of various vegetable oils (on dry matter basis). In contrast, the inclusion of high oil levels (> 5%) in ruminant diets reduced voluntary DMI (Bessa et al., 2005; Francisco et al., 2015) due to the associated increase in dietary energy density (Allen, 2000) and potential reduction in feed palatability and impairment of digestive nutrient flows (Ikwuegbu and Sutton, 1982; Annett et al., 2011).

The absence of treatment effects on DMI was expected due to low levels of oil supplementation (at or below 50 g/kg DMI) (Byers and Schelling, 1988; Wachira et al.,

2000), the similarity in ME content and the forage to concentrate ratio between the diets. Waldo (1986) pointed out that DMI is negatively correlated with dietary NDF content, when rumen fill limits voluntary feed intake. In the present study, the NDF content of experimental diets was similar.

The inclusion of up to 5% canola oil or flaxseed oil in lamb diets did not negatively influence the final LWT, ADG, FE, the changes in LWT, body conformation and BCS, which can be attributed to the similarity in DMI across treatments, the moderate level of oil supplementation in our study, and the fact that the experimental diets were isoenergetic and isonitrogenous. The results are in agreement with previous studies by Malau-Aduli et al. (2014) in purebred Merino and crossbred prime lambs supplemented with one kg pellets and at the same levels of canola oil as used in this study, and by Meale et al. (2015) who fed Canadian Arcott lambs with 2% canola, flaxseed or safflower oil. Radunz et al. (2009) also concluded that blended soybean and flaxseed oil inclusion in finishing diets did not influence lamb growth performance.

Apart from meat production, wool products also contribute a significant percentage to economic returns in the dual-purpose sheep enterprise (Holman and Malau-Aduli, 2014). Wool is not a uniform biological product because its physical characteristics vary depending on sheep genetics, environment and management strategies (Warn et al., 2006). Wool fibres are primarily composed of protein (Plowman, 2003), thus wool synthesis is frequently influenced by the quality and amount of dietary protein, especially sulphur-containing amino acids – cysteine and methionine (Khan et al., 2012; Holman and Malau-Aduli, 2014). However, the inclusion of canola oil or flaxseed oil did not alter the CP content in lamb diets. This could partly explain the fact that wool quality remained unchanged during the 10 weeks of oil supplementation. Additionally, the absence of

significant differences in wool quality could be due to similarity in protein sources, the forage to concentrate ratio and DMI between the dietary treatments. This observation is in agreement with a previous study (Malau-Aduli et al., 2014), in which wool quality was not impacted when canola oil was used as a supplement in lamb finishing diets.

Regarding gender effects, the difference in WH gain between ewes and wethers in the present study was consistent with the findings of previous work (Holman et al., 2012, 2014a). These differences may be attributed to variation in body sizes between genders (Lewis et al., 2006). Sowande and Sobola (2008) and Cake et al. (2007) demonstrated that ewes have a lower size at maturity compared with male lambs because of the high concentrations of oestrogen restricting the growth of the long bones including limb bones. Pitchford (1992) indicated that ewes would be expected to have less CFY and FD than intact rams because they have less surface area, although the amount of follicles are similar between genders in the same breed which results in a greater follicle density. However, the findings were contrary, in our study gender did not affect the wool traits except for FSD which may be due to hormonal effects which impact on metabolic pathways (Holman et al., 2014b). Malau-Aduli and Akuoch (2012) also stated that wethers have lower circulating concentrations of testosterone than intact rams and this can influence the wool traits.

The differences in DMI and CG gain between breeds in this study were in agreement with those reported in other studies (Holman et al., 2012, 2014a). Furthermore, the wool quality differences between purebred Merinos and crossbred lambs in this study were consistent with the findings of Holman et al. (2014b) who found purebred Merino lambs had lower FD, FC, SF and higher CF compared with crossbred-Merino lambs. Scales et al. (2000) also concluded purebred Merinos had lower FD and higher CFY than crossbred-Merino lambs. A possible explanation for these differences includes variation in genetic disposition

towards muscle growth, wool growth or body fat deposition (Mitchell, 2007; Rodríguez et al., 2011) and the diversity in production type between breeds (Ekiz et al., 2009). The three breeds chosen in this study represented a variety of common genotypes in the Tasmanian and Australian sheep industry. Purebred Merino lambs represent a breed typically selected for wool production, but also frequently used as a maternal breed in crossbred prime lamb production, whereas crossbred WxC lambs are more representative of commercial prime lamb production. Crossbred CxM lambs represent dual-purpose prime lamb production systems (Swan et al., 2007). Lewis et al. (2006) exemplified that lambs from high growth breeds grew larger than their counterparts from low growth pedigrees. Another possible explanation for differences in wool quality traits is the variation in wool follicle density between breeds (Adams and Cronjé, 2003). Various studies have supported the observation that lamb breed is a significant source of variation in growth performance (Rodríguez et al., 2011; Holman et al., 2014a; Holman et al., 2014b; Gardner et al., 2015).

The significant interactions between nutrition and breed on FE and lamb growth traits detected in our study were widely acknowledged in published literature (Hegarty et al., 2006a; Van Beem et al., 2008; Holman et al., 2014a). These interactions provide lamb producers with a wider range of choices of nutritional regimen and breed combinations for targeting the reduction of feed cost and the optimal attainments of slaughter weights (Holman et al., 2014a). The nutritional and genetic interactions for lamb growth traits may allow the industry to develop lamb production strategies to suit a spectrum of market specifications (Hegarty et al., 2006a).

The correlations between wool quality traits in the present study are in accordance with preceding published literature (Safari et al., 2005; Holman et al., 2014b). The very strongly positive relationship between FD and SF is logical and expected because SF is refined from

FD and CV (Holman and Malau-Aduli, 2012). Comfort factor is used to describe the percentage of fibres with FD less than 30 μm (Malau-Aduli and Akuoch, 2012). It means that increasing CF is attributable to a decrease in FD. Hence, Malau-Aduli and Akuoch (2010) concluded that there were highly negative correlations between CF and both FD and SF. Safari et al. (2005) reported that the two measurements of variation in FD (FSD and CV) had strongly positive correlations, although there was a moderate correlation between FD and FSD. These findings are in line with the results in this study.

3.6. Conclusions

Supplementing pellets containing up to 5% canola oil or flaxseed oil in dual-purpose prime lambs had no negative effect on DMI, growth performance and wool quality. Moreover, the inclusion of 5% canola oil in pellets increased CG gain. Lamb breed significantly affected DMI, CG gain and wool quality. First-crossbred WxC lambs had the greatest DMI and CG gain, while purebred Merinos had the best wool quality. Ewes had significantly less WH gain and greater FD than wethers, although gender did not influence other characteristics. Oil supplementation and breed interactions on FE and lamb growth traits were observed. Moderate to very strong correlations detected between wool quality traits were significant. In conclusion, canola and flaxseed oils can be effectively used in dual-purpose sheep systems during 10 weeks of feedlot period. Additionally, the observed interaction effects of breed with oil supplementation permit flexibility in operational options of optimising profitability from meat in the dual-purpose prime lamb production. It is proposed that supplementing 5% canola oil in CxM lamb diets or 5% flaxseed oil in WxC lamb diets could considerably improve their feed efficiency and growth performance without detrimental impacts on wool quality.

Chapter 4: Growth performance and carcass characteristics of Australian prime lambs supplemented with pellets containing canola oil or flaxseed oil

4.1. Abstract

The objective of this study was to investigate the effects of enriched omega-3 oil supplemental pellets, breed and gender on lamb liveweight (LWT), body conformation and carcass characteristics, and to assess the relationships between body conformation and growth under an intensive finishing condition. Sixty ewe and wether prime lambs seven-month-old were randomly allocated to one of five dietary treatments: no oil inclusion (Control); 2.5% canola oil; 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil, balanced by breed (purebred Merinos (MxM) and Corriedale x Merino (CxM) and White Suffolk x Corriedale (WxC) first crosses). Lambs were individually supplemented with one kg pellets per day and had free access to lucerne hay and water throughout the 7-week feeding trial, after a 3-week adaptation. Dietary oil inclusion did not cause significant differences in daily feed intake, growth performance and carcass characteristics ($P > 0.05$). However, first-cross WxC lambs had significantly greater feed intake, chest girth and body conformation score ($P < 0.05$) than MxM and CxM lambs. Carcass weight, dressing percentage and fat depth of crossbred lambs were significantly greater than those of MxM ($P < 0.05$). Significant interactions between oil inclusion and breed on average daily gain (ADG) and feed conversion ratio were observed. There were positive and highly significant correlations between LWT, ADG and body conformation measurements ($P < 0.01$). These findings suggest that prime lamb producers can better manage and match their breeding goals with feed resources by supplementing first-cross CxM lambs with pellets containing

5% canola oil or feeding first-cross WxC lambs with 5% flaxseed oil pellets during the 10-week intensive finishing period.

4.2. Introduction

Red meat has been linked to a high risk of cardiovascular diseases in humans (Jerónimo et al., 2009; Mozaffarian and Wu, 2011), because it contains high levels of saturated (SFA) and low levels of polyunsaturated (PUFA) fatty acids (Jerónimo et al., 2012; Pereira and Vicente, 2013). Enser et al. (1996) reported that lamb contained a greater fat percentage than most other animal protein sources and lower levels of PUFA in comparison with seafood or pork. However, it may be a good dietary source of healthy fatty acids such as conjugated linoleic acid and long-chain polyunsaturated fatty acids (LC-PUFA) (Jerónimo et al., 2009). It has been reported that the PUFA content can be improved by supplementing PUFA-rich plant oils or fish oil in lamb finishing diets (Ferreira et al., 2014; Francisco et al., 2015). Canola and flaxseed oils contain an abundance of α-linoleic acid (ALA, 18:3n-3) (Gillingham et al., 2011; Ding et al., 2017) which serves as a potential precursor for the microbial synthesis of the more potent n-3 LC-PUFA (Robert et al., 2005). Many studies have comprehensively used these oils to improve the PUFA content of lamb meat (Jerónimo et al., 2009; Urrutia et al., 2015; Flakemore et al., 2017). However, the impacts of oil supplementation on animal growth, body conformation and carcass characteristics, which are of utmost economic importance, have received little attention (Annett et al., 2011) and even more limited on-farm research has been conducted on the appropriate supplementary levels and optimal duration of feeding these oils to prime lambs.

Due to low wool prices in the last two decades (Rowe, 2010; Malau-Aduli and Akuoch, 2012), meat production from prime lamb has been of increasing interest in the Australian sheep industry (Berry, 2016) because of the high market demand. However, the Australian

national flock remains Merino-dominant, a specialised wool breed (Hatcher et al., 2010). In order to obtain fast-growing lambs, the practice of crossbreeding Merino ewes with meat-type terminal sires, or producing prime lambs from purebred and crossbred meat breeds is now more frequent in the sheep meat industry. The intensive supplementation of prime lambs with commercial concentrates during feedlot (finishing) is also a common practice. A major on-farm project, which comprehensively evaluated a wide range of Australian prime lamb breeds, concluded that purebred Merinos had lower pre-slaughter weight, hot carcass weight (HCW) and dressing percentages (DP) than Terminal and Maternal sired lambs (Gardner et al., 2015). The effects of breed on lamb growth performance (Holman et al., 2012) and carcass attributes (Hegarty et al., 2006a; Ponnampalam et al., 2007; Walkom and Brown, 2017) have been extensively studied across Australia. However, these studies were entirely conducted under grazing conditions with seasonal variability in pasture supply and without investigating associated differences in body conformation. Corriedale and White Suffolk crossbred lambs are a significant component of the dual purpose breeds that constitute approximately 30% of the prime lamb industry that meets the demands of customers seeking high quality, lean and tender lamb. These crossbreds are not only dominant in the Australian meat industry, but also part of an innovative lamb marketing program, known as Island Prime, a unique Tasmanian-branded product that meets tight specifications based on eye muscle shape, carcass weight and fat score. Studies comparing growth and carcass characteristics between sheep breeds in intensive sheep production systems remain limited. Therefore, the objective of the present study was to investigate growth performance, feed efficiency, body conformation and carcass characteristics of Australian prime lambs from three different breeds supplemented with either elevated levels of canola oil or flaxseed oil pellets in an intensive finishing

management system, and to estimate phenotypic correlations between body conformation and growth traits under the same management system.

4.3. Materials and methods

This study was conducted at the Tasmanian Institute of Agriculture's Research and Demonstration Station, Burlington Road, Cressy, Tasmania, Australia, between June and August 2014. All lambs were cared for following the 2013 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. All procedures were approved by the University of Tasmania Animal Ethics Committee (Permit Number A13839).

4.3.1. Animals, diets and experimental design

Sixty weaner ewe and wether prime lambs, 7 months of age, were randomly selected and assigned to a completely randomized experimental design with an average LWT of 33.4 ± 0.7 kg and body condition score of 2.7 ± 0.3. The experimental lambs comprised 20 purebred Merinos (MxM), 20 first-crosses from Corriedale sires mated to Merino dams (CxM), and 20 progeny of White Suffolk sires mated to Corriedale dams (WxC). They were randomly allocated to one of five isocaloric and isonitrogenous dietary treatments (12 lambs per treatment), balanced by breed. Each lamb was daily supplemented with one kg wheat-based concentrate pellets, with or without oil inclusion. The treatments were: no oil inclusion (Control); 2.5% canola oil (DM basis); 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil. The study lasted for 7 weeks following a three-week adaptation period. Lambs were raised in individual pens and offered their daily experimental ration at 09.00 hours. The lambs also had unlimited access to lucerne hay and clean water. Feed offered and refusals were recorded daily and samples were collected for subsequent analyses.

4.3.2. Liveweight, body conformation and feed conversion ratio

All measurements were assessed by the same researcher throughout the trial while lambs were restrained and in a relaxed state, with heads comfortably erect and standing stably upon all four legs on flat ground to minimize variation and stress. Liveweights were measured using a calibrated electronic scale (Ruddweigh 3000XT; Gallagher Group, Hamilton, New Zealand). Liveweight and body conformation traits were measured on days 0, 21, 35 and 49 of the experiment before each lamb received its daily ration. The body conformation measurements were taken using a measuring tape; chest girth (CG) as body circumference just behind the lamb's forelegs (Holman et al., 2012); wither height (WH) as the span between the highest peak over the scapulae; and body length (BL) as the distance between the base of the neck and the tuber ischia or "pin" bone (Shehata, 2013). Body condition scores (BCS) were also subjectively determined on a scale ranging from 0 (emaciated) to 5 (obese) by feeling the layer of fat on the back muscle using the thumb and fingers on the ribs as described by McLeod et al. (2010). Feed conversion ratio was computed as per Flakemore *et al.* (2017).

4.3.3. Carcass measurements

At the end of the feeding trial, all lambs were fasted overnight for 10 hours and transferred the following morning to a commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania) adjacent to the experimental site. They were electrically stunned and humanely slaughtered the next day in accordance with Meat Standards of Australia guidelines. Hot carcasses weights (HCW) were measured immediately after slaughter and the removal of non-carcass components (head, hide, intestinal tract, and internal organs). Dressing percentage (DP) was calculated as: DP (%) = (HCW/LWT) x 100. Carcasses were chilled under commercial conditions at 4°C. After 12 h of chilling, cold carcass weights (CCW) were measured and chilling loss (CL) was computed as the difference between hot carcass

and cold carcass weights. Thereafter, the *Longissimus thoracis et lumborum* (LTL) muscle was sampled at the 12/13th rib from left side of each carcass within 24 h post-mortem. Using a GR fat knife, fat depth (FDe) was measured at the C site (depth of fat over the maximum depth of loin muscle at the 12/13th rib). Body wall thickness (BWT) was determined at the GR site (11 cm from the midline of carcass at the 12/13th rib). Ribeye area (REA) was measured using a clear plastic grid to determine the cross-section of the LTL muscle at the 12/13th rib. Percentage boneless, closely trimmed retail cuts (BCTRC) was calculated using the equation: %BCTRC = (49.936 – (0.0848 x 2.205 x HCW) - (4.376 x 0.3937 x FDe) – (3.53 x 0.3937 x BWT) + (2.456 x 0.155 x REA) (Neville et al., 2010).

4.3.4. Feed chemical composition and fatty acid analysis

Supplementary pellet and lucerne hay samples were pooled and ground through a 1-mm screen. They were dried in a fan-forced oven at 65°C to a constant weight to determine dry matter (DM) content. Total Nitrogen (N) was quantified using an elemental analyzer (PE2400 Series II; Perkin-Elmer Corp, USA) and multiplied by 6.25 to estimate crude protein (CP) content. Ether extract (EE) was determined using an ANKOM15 fat/oil extractor (ANKOM Technology, USA). The contents of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using an ANKOM220 fiber analyzer (ANKOM Technology, USA). Ash content was quantified by combusting the samples in a furnace at 550°C for 5 hours. Organic matter (OM) was computed as OM = 100 – Ash. Non-fibrous carbohydrates (NFC) was calculated as NFC = 100 − (CP + NDF + EE + Ash) (Mertens, 2002). A near infrared reflectance spectroscopy method was used to estimate metabolisable energy (ME) (Garnsworthy and Unal, 2004).

For determination of fatty acid composition, the detailed protocol was previously described by Malau-Aduli et al. (2016). In brief, total lipids in 1 g of feed were solvent extracted

using a modified Bligh and Dyer (1959) procedure. CH_2Cl_2:MeOH:Milli-Q H_2O (1:2:0.8 v/v) was used in a single-phase overnight process to extract sample lipids, followed by phase separation with CH_2Cl_2:saline Milli-Q H_2O (1:1 v/v), and then rotary evaporated at 40^0C to obtain total lipids. Fatty acid methyl esters (FAME) formed by direct methylation of an aliquot of each extracted lipid were three-time extracted with C_6H_{14}:CH_2Cl_2 (4:1 v/v). Internal injection reference standard (C19:0) was added in a 1500 µL vial containing the extracted and reduced under nitrogen gas FAME. A 7890B gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an Equity™-1 fused silica capillary column (15 m x 0.1 mm internal diameter and 0.1-µm film thickness) (Supelco, Bellefonte, PA, USA) using helium as the carrier gas, a flame ionisation detector, a split/splitless injector and an Agilent Technologies 7683B series autosampler were used to analyse the samples. The initial oven temperature of 45°C was held for 1 min, followed by temperature programming at 30°C per min to 140°C, then at 3°C per min to a maximum of 310°C where it was held for 12 min. Individual fatty acid peaks were quantified by ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Fatty acid composition were qualitatively calculated from FA area output: FA (g/100g total fatty acids) = (area of individual FA)*(100)/(total FA area).

4.3.5. Statistical analysis

The Statistical Analysis System software version 9.2 (SAS Inst., Cary, NC) was used to analyse all experimental data. Average daily weight gains (ADG) of each lamb were determined by linear regression of the individual LWT data from the commencement to the end of the feeding trial. Summary descriptive statistics including means and standard errors of mean were calculated and scrutinised for any erroneous data input. The data were subjected to analysis of variance using a General Linear Model (PROC GLM) with oil

supplementation, breed and their interactions fitted as fixed effects and feed intake, LWT, ADG, body conformation and carcass traits as dependent variables. Significant differences and mean separations at the $P < 0.05$ threshold were performed using Tukey's probability pairwise comparison tests. Pearson's correlation coefficients (PROC CORR) between LWT, ADG and body conformation traits were also estimated and significance established using Bonferroni probability pairwise test.

4.4. Results

4.4.1. Diets

The supplemental pellet ingredients and chemical composition of the dietary treatments are presented in Table 4.1. The major carrier ingredient in the pellets was wheat (465 - 551 g/kg). The CP, EE and other chemical composition of the five pellets were relatively similar. The basal diet of lucerne hay had more CP, NDF, ADF and less EE than the pellets. The ME contents were also similar between the five oil supplementary pellets and ranged from 10.8 MJ/kgDM to 11.1 MJ/kgDM.

Table 4.1. Experimental pellet ingredients and chemical composition of feedstuff

Item	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
Ingredients, g/kg as-fed						
Wheat	513	537	545	551	465	–
Paddy rice	260	230	210	220	280	–
Lupins	170	151	138	147	148	–
Canola oil (ml/kg)	-	25	50		-	–
Flaxseed oil (ml/kg)	-		-	25	50	–
Salt	10	10	10	10	10	–
Limestone	21	21	21	21	21	–
Sheep premix	1	1	1	1	1	–
Ammonium sulfate	12.6	12.6	12.6	12.6	12.6	–
Acid buffer	6.2	6.2	6.2	6.2	6.2	–
Sodium bicarbonate	6.2	6.2	6.2	6.2	6.2	–
Dry matter (%)	89.8	90.2	87.9	90.5	89.4	89.6
Chemical composition (%)						
Crude protein	14.7	14.5	14.4	14.5	14.5	17.4
NDF	23.8	23.5	23.9	23.7	23.3	46.5
ADF	9.2	9.3	8.9	9.5	9.0	30.9
NFC[1]	50.5	49.9	47.8	50.5	50.7	27.4
Ether extract	4.5	4.6	4.9	4.7	5.0	1.5
Ash	8.0	7.5	8.2	7.1	6.4	7.2
ME[2], MJ/kgDM	10.8	10.9	11.1	10.8	11.0	9.8

[1]NFC = non-fibrous carbohydrates [NFC = 100 − (CP + NDF + EE + ash)],

[2]ME = metabolisable energy.

The feed FA composition are given in Table 4.2. The principal FA in the pellets were 18:2n-6 and 18:1n-9, while 18:3n-3 and 16:0 accounted for the greatest FA proportions in lucerne hay. Inclusion of oils in the pellets increased 18:3n-3 concentration and the SFA/PUFA ratio. Oil supplementation also increased the level of total n-3 PUFA, but reduced total n-6 PUFA concentration in the pellets. As a consequence, the enriched oil pellets had a lower n-6/n-3 ratio than the control pellets. Lucerne hay had low n-6/n-3 ratio (0.5). In the experimental feeds, n-3 LC-PUFA were not detected.

Table 4.2. Fatty acid composition (g/100g total fatty acids) of diets

	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
14:0	0.2	0.5	0.6	0.2	0.2	0.6
15:0	0.1	0.1	0.1	0.1	0.1	0.4
16:0	18.2	16.9	16.5	19.1	19.8	29.6
17:0	0.1	0.1	0.2	0.1	0.1	0.7
18:2n-6	43.4	28.4	26.7	25.6	24.7	14.9
18:3n-3	3.5	3.6	4.3	4.9	7.2	31.1
18:1n-9	23.9	37.5	38.9	32.3	34.1	2.5
18:0	3.4	4.1	4.1	4.4	5.1	4.7
20:4n-6	ND[1]	ND	ND	ND	ND	ND
20:5n-3	ND	ND	ND	ND	ND	ND
20:3n-6	0.3	0.4	0.4	0.4	0.5	0.4
20:4n-3	0.4	0.5	0.2	0.5	0.6	0.5
20:2n-6	0.1	0.1	0.2	0.1	0.1	0.1
20:0	0.5	0.8	0.7	0.7	0.8	1.5
22:5n-6	ND	ND	ND	ND	ND	ND
22:6n-3	ND	ND	ND	ND	ND	ND
22:5n-3	ND	ND	ND	ND	ND	ND
∑SFA[2]	24.1	23.0	25.0	26.7	28.7	42.4
∑MUFA[3]	27.5	42.6	43.3	36.3	37.4	8.4
∑PUFA[4]	48.4	34.4	31.7	37.0	33.8	49.3
SFA/PUFA	0.5	0.7	0.8	0.7	0.8	0.9
∑n-3 PUFA[5]	3.9	4.1	4.8	5.5	7.9	31.6
∑n-6 PUFA[6]	43.8	28.8	27.4	26.0	25.3	15.3
n-6/n-3	11.1	7.0	5.7	4.7	3.2	0.5

[1]ND: not detected; [2]∑SFA: total saturated fatty acids; [3]∑MUFA: total monounsaturated fatty acids; [4]∑PUFA: total polyunsaturated fatty acids includes; [5]∑n-3 PUFA: total omega-3 PUFA; [6]∑n-6 PUFA: total omega-6 PUFA

4.4.2. Growth rate and feed intake

Daily feed intake was significantly affected ($P < 0.01$, Table 4.3) by lamb breed. First-cross WxC lambs had significantly greater daily feed intake (1.58 kg DM) than MxM and CxM lambs (1.51 kg DM and 1.52 kg DM respectively). However, no differences in LWT changes and feed conversion ratio were observed between the three lamb breeds ($P > 0.05$). Oil supplementation did not affect the growth rate, daily feed intake and feed conversion ratio of experimental lambs ($P > 0.05$). Significant interaction effects on ADG and feed conversion ratio were detected ($P < 0.05$; Table 4.3 and Figure 4.1).

Table 4.3. Effects of oil supplementation and breed on animal performance and interactions (the significant interactions for average daily gain and feed conversion ratio are described in Figure 4.1)

	Treatment					Breed[1]			SEM[2]	P-value[3]		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		Treatment	Breed	Treatment x Breed
Initial liveweight (kg)	35.6	37.1	35.5	36.4	36.0	35.7	36.0	36.5	0.30	0.492	0.466	0.642
Final liveweight (kg)	44.3	46.1	45.3	45.2	45.8	44.4	45.6	46.0	0.36	0.514	0.189	0.171
Average daily gain (kg)	0.182	0.189	0.205	0.184	0.204	0.183	0.200	0.195	0.004	0.067	0.096	0.023
Daily pellet intake (kg DM)	0.80	0.82	0.81	0.82	0.83	0.81[b]	0.80[b]	0.85[a]	0.01	0.496	0.006	0.269
Daily lucerne hay intake (kg DM)	0.73	0.69	0.71	0.72	0.73	0.70	0.72	0.73	0.01	0.298	0.070	0.443
Daily feed intake (kg DM)	1.53	1.51	1.53	1.56	1.55	1.51[b]	1.52[b]	1.58[a]	0.01	0.397	0.002	0.159
Feed conversion ratio (kg feed DM/kg live-weight gain)	8.5	8.2	7.6	8.6	7.7	8.3	7.8	8.3	0.15	0.071	0.174	0.014

[1]MxM, purebred Merino; CxM, Corriedale x Merino; WxC, White Suffolk x Corriedale.

[2]SEM, standard error of the mean.

[3] Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$).

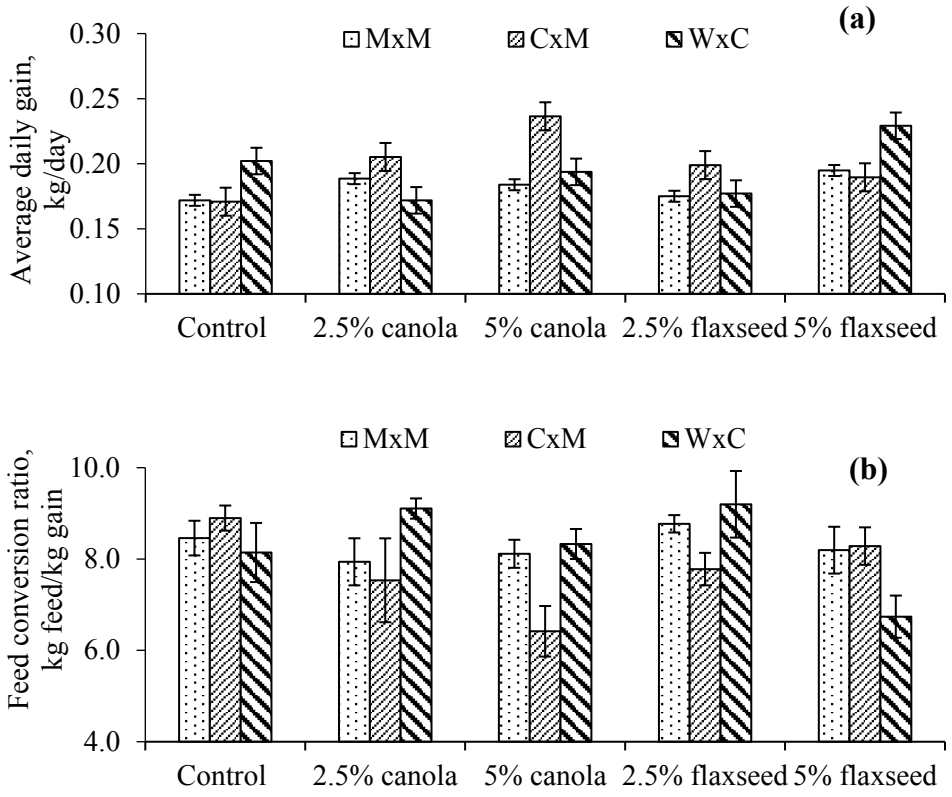

Figure 4.1. Interaction effects on: a) average daily gain (ADG) and b) feed conversion ratio of experimental lambs (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale; bars are standard errors of the mean).

First-crossbred CxM lambs recorded the greatest ADG (0.236 kg) when they were fed pellets containing 5% canola oil, while the greatest ADG of WxC lambs was observed in the 5% flaxseed oil treatment (0.229 kg) (Figure 4.1a). In contrast, feed conversion ratios were lowest in CxM lambs fed 5% canola oil pellets (6.4 kg feed/kg gain) and WxC lambs offered pellets containing 5% flaxseed oil (6.7 kg feed/kg gain) (Figure 4.1b).

Table 4.4. Variation in experimental lamb body conformation as affected by oil supplementation, breed and interaction

	Treatment					Breed[1]			SEM[2]	P value[3]		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		Treatment	Breed	Treatment x Breed
Initial chest girth (cm)	80.1	81.3	80.3	80.9	81.7	80.5	80.6	81.6	0.26	0.149	0.141	0.501
Final chest girth (cm)	90.5	92.3	93.8	90.4	91.8	90.6^{b}	90.6^{b}	94.1^{a}	0.49	0.074	0.001	0.221
Initial body length (cm)	63.6	65.6	64.6	64.2	63.9	64.6	64.0	64.7	0.30	0.185	0.550	0.584
Final body length (cm)	71.0	70.6	70.9	70.7	71.1	71.3	70.7	70.6	0.29	0.977	0.531	0.171
Initial wither height (cm)	59.3	59.7	60.0	59.5	59.3	60.2	59.7	58.8	0.28	0.913	0.128	0.192
Final wither height (cm)	66.0	65.9	66.4	65.6	65.3	66.8	66.0	65.9	0.23	0.403	0.941	0.370
Initial BCS[4]	2.9	3.0	3.0	3.1	2.8	3.0	2.9	3.0	0.04	0.251	0.586	0.480
Final BCS	3.3	3.5	3.6	3.4	3.5	3.4^{b}	3.4^{b}	3.6^{a}	0.04	0.187	0.043	0.887

[1, 2, 3] are as indicated in Table 4.2.

[4]BCS, body condition score (ranked from 0 (emaciated) to 5 (obese))

4.4.3. Body conformation traits

Final CG and BCS were significantly influenced ($P < 0.05$, Table 4.4) by lamb breed. First-cross WxC lambs had markedly greater final CG (94.1 cm) than purebred Merinos (90.6 cm) and CxM lambs (90.7 cm). Furthermore, the final BCS of WxC lambs (3.6) was significantly greater than that of purebred Merino and CxM lambs (3.2). However, lamb breed did not affect BL and WH ($P > 0.05$). The inclusion of oil in the pellets did not result in significant differences ($P > 0.05$) in body conformation. No significant interaction effect on body conformation traits was detected.

4.4.4. Phenotypic relationships between growth and body conformation traits

There were positive relationships between lamb growth and body conformation measurements (Table 4.5). The correlation between ADG and WH (0.39) was highly significant ($P < 0.01$). Other correlations between LWT, ADG, CG, BL, WH and BCS were very highly significant ($P < 0.001$). The relationships between ADG and body conformations were moderate, ranging from 0.39 to 0.55. Moderate to high correlations between LWT and body conformation traits were observed (0.62 – 0.84) with the strongest relationship between LWT and CG.

4.4.5. Carcass characteristics

Lamb breed significantly affected ($P < 0.05$) carcass weights, DP and FDe (Table 4.6). In particular, crossbred lambs had markedly heavier HCW and CCW than purebred Merinos ($P < 0.05$), but there was no carcass weight difference between CxM and WxC lambs ($P > 0.05$). Similarly, purebred Merino lambs recorded the smallest DP and FDe, while DP and FDe in the CxM and WxC crossbreds did not significantly differ. However, there was

no significant difference in CL, BWT, REA and BCTRC between the three breeds of lambs. Carcass characteristics were unaffected by oil supplementation. Interaction effects on carcass characteristics were not significant (P > 0.05; Table 4.6).

Table 4.5. Pearson's correlation coefficients between liveweight, ADG and body conformation measurements in genetically different prime lambs

	Body length	Wither height	BCS[1]	Liveweight	ADG[2]
Chest girth	0.68***[3]	0.60***	0.60***	0.84***	0.55***
Body length		0.57***	0.54***	0.77***	0.53***
Wither height			0.41***	0.62***	0.39**
BCS				0.68***	0.46***
Liveweight					0.63***

[1]BCS, body condition score.

[2]ADG, average daily gain.

[3]Level of significance: ** highly significant (P < 0.01), *** very highly significant (P < 0.001)

Table 4.6. Oil supplementation, breed and interaction effects on lamb carcass characteristics

	Treatment					Breed[1]			SEM[2]	P-value[3]		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		Treatment	Breed	Treatment x Breed
Hot carcass weight (kg)	20.9	22.0	22.0	21.7	21.9	20.9[b]	22.1[a]	22.2[a]	0.21	0.401	0.012	0.477
Cold carcass weight (kg)	20.4	21.4	21.4	21.1	21.3	20.3[b]	21.5[a]	21.7[a]	0.20	0.389	0.011	0.491
Dressing percentage (%)	47.4	47.7	48.6	48.0	47.8	46.9[b]	48.3[a]	48.3[a]	0.16	0.061	0.001	0.186
Chilling loss (%)	2.8	2.7	2.7	2.7	2.7	2.8	2.7	2.7	0.04	0.803	0.147	0.252
Fat depth (mm)	4.5	4.6	4.4	4.8	4.8	3.9[b]	4.9[a]	5.2[a]	0.18	0.932	0.008	0.175
Body wall thickness (mm)	16.2	17.1	18.5	17.9	18.1	16.1	18.2	18.4	0.46	0.535	0.100	0.906
Ribeye area (cm^2)	12.2	12.4	12.8	12.5	12.8	12.2	12.5	13.0	0.15	0.560	0.134	0.279
BCTRC[4] (%)	49.8	49.6	49.6	49.6	49.6	49.5	49.9	49.5	0.09	0.962	0.048	0.094

[1, 2, 3] are as indicated in Table 4.2.

[4]BCTRC, Boneless, closely trimmed retail cuts.

4.5. Discussion

Canola and flaxseed oils contain an abundance of α-linoleic acid (ALA, 18:3n-3) (Gillingham et al., 2011; Ding et al., 2017) which serves as a potential precursor for the synthesis of the more potent n-3 LC-PUFA (Robert et al., 2005) as depicted in the fatty acid composition of the experimental diets. Many studies have comprehensively used these oils to improve the PUFA content of lamb meat (Jerónimo et al., 2009; Urrutia et al., 2015; Flakemore et al., 2017)

Generally, the inclusion of high levels (> 5% dry matter basis) of oil in ruminant diets tends to decrease voluntary feed intake (Bessa et al., 2005; Francisco et al., 2015) because of the associated increase in dietary energy density (Allen, 2000) and potential reduction in feed palatability (Annett et al., 2011) and digestive nutrient flows (Ikwuegbu and Sutton, 1982). However, in the present study, lambs receiving oil in their diets did not show any decrease in total feed intake, which is consistent with other previous studies reported by (Manso et al., 2009; Meale et al., 2015) that did not observe any decline in feed intake when 2 – 5% inclusion levels of various vegetable oils were added to lamb diets. The absence of any significant effect of oil inclusion on feed intake was expected due to the similarity in ME content and lucerne hay to concentrate ratio between the diets. In contrast, Ferreira et al. (2014) reported a reduction in feed intake when Santa Ines lambs were supplemented with 4% soybean oil in their diets. However, the diets used in their study were not isoenergetic, and the lambs reached the same energy intake and ADG levels with decreased feed intake. Variable results from other ruminant supplementation studies have been reported on the effects of dietary oil supplementation on growth performance and carcass characteristics (Meale et al., 2015; Adeyemi et al., 2016b; Ghafari et al., 2016). Such variability could be due to differences in duration, levels and nature of oil inclusion (Manso et al., 2009; Francisco et al., 2015) which directly influenced the populations and activities of rumen microbes (Wachira et al., 2000; Wanapat et al., 2011). It could also be associated with variation in dietary composition including the level of concentrate,

crude protein content and energy density of the experimental diets. Our results herein demonstrated that oil supplementation did not alter lamb LWT, body conformation and carcass traits. The absence of significant differences in animal performance and carcass measurements could partly be attributed to the level of oil supplementation in our study (⩽5%), the similarity in daily feed intake across treatments and the fact that the experimental diets were isoenergetic and isonitrogenous. The outcomes are in accordance with previous studies by Malau-Aduli et al. (2014) in purebred Merino and crossbred prime lambs supplemented with the same quantity of pellets and levels of canola oil as this trial and by Meale et al. (2015) who fed Canadian Arcott lambs with 2% canola, flaxseed or safflower oil. Radunz et al. (2009) also concluded that soybean and flaxseed oil inclusion in finishing diets did not influence lamb growth performance and carcass characteristics. Several works reported that dietary fat levels at or below 50 g/kg DM will not result in any detrimental impact on LWT, ADG and carcass traits (Badee and Hidaka, 2014; Ferreira et al., 2014). Therefore, our outcomes, in combination with previous studies, indicate that the inclusion of up to 5% canola or flaxseed oil in finishing diets has no detrimental effects on lamb growth and carcass measurements.

The variation in daily feed intake between lamb breeds in the present study is in agreement with the works of Wildeus et al. (2007) and Ríos et al. (2011) who investigated various hair lamb breeds. Similarly, Cammack et al. (2005) and Snowder and Van Vleck (2003) concluded that under feedlot conditions, daily feed intake in growing lambs demonstrates sustainable genetic variation. In beef cattle, Crowley et al. (2010) and Schenkel et al. (2004) also found significant breed effects on feed intake. This variation could be due to differences in gastro-intestinal tract capacity (Dillon et al., 2003; Boujenane, 2015) and diversity in daily pellet intake between breeds.

The lower final CG and BCS in purebred Merinos compared to crossbred lambs in the present study was consistent with those reported in other works (Holman et al., 2012; Malau-Aduli et

al., 2012b; Holman et al., 2014a). Furthermore, the differences in some carcass measurements between crossbred lambs and purebred Merinos were in line with Ekiz et al. (2009) and Burke et al. (2003), who compared the carcass measurements of five different lamb breeds under intensive feeding systems. These may be attributed to additive gene effects and heterosis in crossbred lambs (Boujenane, 2015). Other possible explanations for these differences include variation in genetic disposition towards muscle growth, body fat deposition or wool growth (Rodríguez et al., 2011) and the diversity in production type between breeds (Ekiz et al., 2009). The three breeds used in this study represented a variety of genotypes used in sheep production systems. Purebred Merino lambs have typically been selected for wool production, but are also frequently used as the maternal breed in prime lamb production (Mortimer et al., 2008). First-cross WxC lambs, on the other hand, represent a terminal breed for commercial prime lamb production. CxM crossbred lambs represent typical dual-purpose breeds in prime lamb production systems (Warn et al., 2006).

Effects of supplementation with oil (Ferreira et al., 2014; Meale et al., 2015) and breed (Burke et al., 2003; Boujenane, 2015) on feed intake and growth responses of lambs have been investigated comprehensively. When either fat inclusion or lamb breed and their interaction were fixed experimental effects Annett et al. (2011), reported no interaction effects on animal intake and growth. Furthermore, Wachira et al. (2002) found a significant interaction between dietary fat supplementation and breed for daily feed intake. However, it was interesting that significant interactions between oil supplementation and lamb breed on ADG and feed conversion ratio were detected in our study. This was in agreement with Hegarty et al. (2006a) and Holman et al. (2014a), who concluded that there were significant interaction effects between nutrition and genetic potential for animal performance. The significant interactions between oil supplementation and lamb breed on ADG and feed conversion are relevant because

they indicate that on the same oil diet, different lamb breeds will convert the feed and gain weight differently with these variations impacting on meat PUFA content.

Positive and highly significant correlations between LWT, ADG and body conformation traits in the present study were in accordance with the findings of Malau-Aduli et al. (2012b) and Holman et al. (2012). From such strong relationships, some studies established LWT estimation equations based on body conformation measurements using multiple regression models (Afolayan et al., 2006; Cam et al., 2010; Yilmaz et al., 2013). Our results in combination with previous findings reaffirm that body conformation measurements can be used as predictors of LWT.

4.6. Conclusions

The addition of canola or flaxseed oil in lamb feeds did not affect feed intake, growth performance and carcass traits. Feed intake, final CG and BCS were significantly influenced by lamb breed, with optimal performances by WxC crossbred lambs. Crossbred lambs had greater HCW, CCW, DP and FDe than purebred Merinos. There were significant interactions between oil supplementation and lamb breed on ADG and feed conversion ratio. The correlations between LWT, ADG and body conformation measurements were positive and highly significant. In conclusion, both canola and flaxseed oils can be effectively used in feedlot regimes in the prime lamb industry without any detrimental effect on animal performance and carcass characteristics. It is hereby suggested that supplementing first-cross Corriedale x Merino lambs with pellets containing 5% canola oil or feeding first-cross White Suffolk x Corriedale lambs with 5% flaxseed oil pellets during the 10-week intensive finishing phase offers the best means of improving average daily gain and reducing feed conversion ratio.

Chapter 5: Fatty acid profiles of muscle and adipose tissues, and meat sensory characteristics of Australian prime lambs supplemented with pelleted canola and flaxseed oils.

5.1. Abstract

The effects of canola or flaxseed oil dietary supplementation on *Longissimus thoracis et lumborum* (LTL) muscle and visceral adipose tissue fatty acid (FA) profiles and meat sensory traits in Australian prime lambs from different breeds were investigated. Sixty lambs were fed one of the following pellet treatments: no oil (Control), 2.5% canola, 5% canola, 2.5% flaxseed and 5% flaxseed, balanced by breed (purebred Merino, and first-cross lambs from Corriedale rams mated to Merino ewes and White Suffolk rams mated to Corriedale ewes). Lambs were individually supplemented daily with one kg of oil-enriched wheat-based pellets throughout the 7-week feeding trial, after a 3-week adjustment period and had unlimited access to water and lucerne hay. At the end of the feeding trial, all animals were slaughtered. From each carcass, an LTL muscle sampled at the 12/13th rib interface and a visceral adipose tissue sample from the liver area were taken and subjected to fatty acid analysis. Another LTL muscle sample was removed and utilised for sensory evaluation of meat eating quality. The inclusion of 5% flaxseed oil significantly decreased the n-6/n-3 ratio in both tissues. The muscle from lambs fed 5% oil supplements had greater omega-3 long-chain polyunsaturated FA (n-3 LC-PUFA) contents and reached the claimable health-benefitting value without deleterious sensory effects. The n-3 LC-PUFA content in visceral adipose tissue was negligible. Tissue FA profiles and sensory quality were influenced by breed. There were significant interactions between oil supplementation and lamb breed on some visceral adipose FA and meat juiciness. These findings indicate that a combination of dietary manipulation and lamb genetics can be used as an effective management tool to deliver nutritionally improved n-3 LC-PUFA lamb to consumers.

5.2. Introduction

Heart disease is a global health threat and the leading cause of death worldwide (Go et al., 2014). It is widely accepted that omega-3 long-chain polyunsaturated fatty acids (n-3 LC-

PUFA) including eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) play a critical role in the prevention of cardiovascular and inflammatory diseases in humans (Mozaffarian and Wu, 2012) and also the improvement of visual and brain development in infants and children (Gould et al., 2013). The n-3 LC-PUFA cannot be synthesised by humans and other vertebrates due to the absence of Δ^{-15} desaturase enzyme, and therefore need to be included in the diet (Innis, 2011). The recommended dietary n-3 LC-PUFA intake for the prevention of chronic diseases in adult males and females in Australia and New Zealand are 610 and 430 mg/day, respectively (NHMRC, 2006). Nichols et al. (2010) indicated that Australian adults need about 500 mg of n-3 LC-PUFA daily, but the average intake of n-3 LC-PUFA in Australia was reported to be 246 mg/day (Howe et al., 2006). Thus, there is clearly a current shortfall in dietary intake of these health-benefitting n-3 LC-PUFA, and a recognized need for an increase in the consumption of n-3 LC-PUFA in the Australian diet.

Meat from ruminants has been implicated in the increased risk of cardiovascular diseases and metabolic syndrome, due to its high content of saturated fatty acids (Pereira and Vicente, 2013). However, ruminant meat is an important source of n-3 LC-PUFA contributing approximately 40% of the average daily intake of these FA for adults in Australia (Clayton, 2014). Cooper et al. (2004) reported that supplementing lambs with fish oil and/or marine algae, which are rich in n-3 LC-PUFA, substantially increased the levels of EPA and DHA in *Longissimus* muscle and adipose tissues. However, the use of marine oils in animal diets may increase fishy flavour and rancidity (Nute et al., 2007), and also may not be sustainable or cost-effective. Recently, vegetable oils, especially oil from canola and flaxseed, have been supplemented in lamb diets as a source of n-3 PUFA to enhance the n-3 LC-PUFA content in their meat (Radunz et al., 2009; Noci et al., 2011). The principal n-3 PUFA in canola oil and flaxseed oil is exclusively α-linoleic acid (ALA, 18:3n-3) (Dubois et al., 2007; Ding et al., 2017). This FA can act as a

potential precursor for the synthesis of the more beneficial n-3 LC-PUFA (Robert et al., 2005). In addition to diet, animal genetics may contribute a long-term and cumulative impact on lamb FA concentration (Demirel et al., 2004; Sinclair, 2007). Therefore, FA profiles of lamb products can theoretically be manipulated through crossbreeding. However, on-farm research on the potential modification of FA profiles in both muscle and visceral adipose tissues from lambs fed canola oil or flaxseed oil and the appropriate supplementary levels of these oils in diets remains limited, hence the need for the current study.

Along with nutritional attributes, sensory characteristics are a key factor strongly influencing the demand and willingness to pay decisions of lamb consumers (Pethick, 2006). Numerous studies with sheep meat have concluded that sensory traits were mainly influenced by animal age (Della Malva et al., 2016) and feeding regimes (De Brito et al., 2016; Erasmus et al., 2016; Girard et al., 2016). However, most of these studies were conducted to compare sensory variation in lambs fed different types of forages. Studies investigating the effects of concentrate-based systems on sensory characteristics are currently limited. Other studies have shown that lamb breed has an impact on meat eating quality (Hopkins and Mortimer, 2014; Pannier et al., 2014a), but this impact is highly variable. For instance, Monaco et al. (2015) reported that sire breed significantly affected sensory traits, while Safari et al. (2001) concluded that no differences occurred in the eating quality for loin meat from purebred Merinos and their crossbred lambs.

Therefore, the major objective of this study was to investigate the FA profiles of LTL muscle and visceral adipose tissues, and variation in sensory traits of meat from Australian prime lambs supplemented with canola oil or flaxseed oil enriched pellets. The following hypotheses were tested: Pellets enriched with canola or flaxseed oils: (1) enhance n-3 LC-PUFA profiles to meet the values considered more optimal for human diets; (2) do not adversely influence sensory properties of the meat, and (3) lamb breed influences tissue FA profiles and meat eating quality.

5.3. Materials and methods

5.3.1. Location, animal ethics permit and experimental animals

This research was conducted at the Cressy Research and Demonstration Station, Cressy, Tasmania, Australia between June and August 2014. The experimental design and procedures were approved (Permit No. A13839) by the University of Tasmania Animal Ethics Committee. The study was also in accordance with the 1993 Tasmania Animal Welfare Act and the 2013 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Sixty weaned prime lambs at seven months of age, with an initial mean liveweight of 33.4 kg and body condition score of 2.7 were utilised in this study. The lambs comprised 20 purebred Merinos (MxM), 20 first-crosses from Corriedale sires mated to Merino dams (CxM), and 20 progenies of White Suffolk sires mated to Corriedale dams (WxC). Animals were dewormed and identified with ear tags prior to the commencement of the feeding trial.

5.3.2. Experimental design and dietary management

The experimental design was completely randomised, with 5 treatments and 12 lambs per treatment, balanced by breed. Lambs were supplemented daily with one kg of wheat-based concentrate pellets, with or without oil inclusion. The treatments included 1) no oil inclusion (Control); 2) 2.5% canola oil; 3) 5% canola oil; 4) 2.5% flaxseed oil and 5) 5% flaxseed oil. All diets were isocaloric and isonitrogenous. The study lasted for 10 weeks including a three-week adaptation period. Lambs were housed in individual pens with *ad libitum* access to lucerne hay and clean water. Residual feed were removed prior to fresh feed being offered to experimental lambs at 0900 hours.

5.3.3. Slaughter and sampling

At the end of the feeding trial, all lambs walked to an adjacent commercial abattoir (Tasmanian Quality Meats, Cressy, Tasmania, Australia) for slaughter according to Meat Standards Australia regulations and specifications after an overnight fast with water available in lairage. Visceral adipose tissue samples were taken immediately after evisceration from the liver. After 24 h chilling, two LTL muscle samples at the 12/13th rib interface were removed from each carcass as commercial loin chops (approximately 200 g) – one chop designated for FA analysis, the other for sensory testing. All samples were vacuum-sealed, code-labelled and stored at -20^{0}C until analyses.

5.3.4. Feed chemical analysis

Representative pellet and lucerne hay samples were collected on days 0, 25 and 49 of the experimental period and kept at -20^{0}C for subsequent analyses. At the end of the experiment, the samples were defrosted, pooled and ground through a 1-mm screen. Samples were dried in triplicates in a fan-forced oven to a constant weight at 65^{0}C to determine dry matter (DM) content. Total Nitrogen (N) was quantified using an elemental analyser (PE2400 Series II; Perkin-Elmer Corp, USA), and multiplied by 6.25 to estimate crude protein (CP) content. Ether extract (EE) was determined using an ANKOM fat/oil extractor (ANKOM^{XT15}; ANKOM Technology, USA). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents were measured using an ANKOM fibre analyser (ANKOM^{220}; ANKOM Technology, USA). Ash content was quantified by combusting samples in a furnace at 550^{0}C for 5 hours. Organic matter (OM) was computed as OM = 100 – Ash. Non-fibrous carbohydrates (NFC) was calculated as NFC = 100 − (CP + NDF + EE + Ash) (Mertens, 2002). A near infrared reflectance spectroscopy method (Garnsworthy and Unal, 2004) was used to estimate metabolisable energy (ME).

5.3.5. Lipid analysis

Total lipid extraction and FA profile analysis were undertaken at the Commonwealth Scientific and Industrial Research Organization (CSIRO) Food Nutrition & Bio-based Products, Oceans & Atmosphere, Hobart, Tasmania, Australia. The procedures were outlined in detail by Malau-Aduli et al. (2016). In brief, total lipids in 1 g of feed and muscle and 0.2 g of visceral adipose tissue samples were solvent extracted using a modified Bligh and Dyer (1959) protocol. CH_2Cl_2:MeOH:Milli-Q H_2O (1:2:0.8 v/v) was used in a single-phase overnight process to extract sample lipids, followed by phase separation with CH_2Cl_2:saline Milli-Q H_2O (1:1 v/v), and then rotary evaporated at 40^0C to obtain total lipids.

Fatty acid methyl esters (FAME) were obtained by transmethylation of an aliquot of each extracted lipid sample, and were extracted with C_6H_{14}:CH_2Cl_2 (4:1 v/v, 3 times). Internal injection standard (19:0) was added in 1500 µL vial containing extracted FAME. A 7890B gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an Equity™-1 fused silica capillary column (15 m x 0.1 mm internal diameter and 0.1-µm film thickness) (Supelco, Bellefonte, PA, USA), a flame ionisation detector, a split/splitless injector, and an Agilent Technologies 7683 B series autosampler was used to analyse the samples. Fatty acid peaks were quantified by ChemStation software (Agilent Technologies, Palo Alto, CA, USA). Gas chromatograph -mass spectrometry (GC/MS) analysis were undertaken to confirm FA identities and was performed using a Thermo Scientific 1310 GC coupled with a TSQ triple quadrupole. Samples were injected using a Tripleplus RSH auto sampler with a non-polar HP-5 Ultra 2 bonded-phase column (50 m x 0.32 mm i.d. x 0.17 µm film thickness). The HP-5 column was of similar polarity to the column used for GC analyses. The initial oven temperature of 45°C was held for 1 min, followed by temperature programming at 30°C per min to 140°C then at 3°C per min to 310°C where it was held for 12 min. Helium was used as the carrier gas. Mass spectrometer operating conditions were: electron impact energy 70 eV; emission current 250 µA, transfer line 310°C; source temperature 240°C; scan rate 0.8 scan/sec

and mass range 40-650 Da. Mass spectra were acquired and processed with Thermo Scientific XcaliburTM software (Waltham, MA, USA).

Fatty acid profiles were expressed as percentage (g/100g of total FA or %) and content (mg/100g of wet tissue). Fatty acid percentages were qualitatively calculated from FA area output: FA% = (area of individual FA)*(100)/(total FA area). Fatty acid contents were computed as FA (mg/100g) = (Total lipid percantage)* 0.916 *([FA%]/100)*1000 (Clayton, 2014), with 0.916 used as a lipid conversion factor (Anderson et al., 1975) as cited by Clayton (2014).

5.3.6. Meat sensory evaluation test

Meat sensory evaluation following the protocol described by Thompson et al. (2005a) was performed on 60 loin chops by an untrained panel of 20 consumers. The samples were tested in two sessions and 10 members per session. Consumers were between the ages of 30 and 60 years, and ate red meat at least twice per week. To become familiar with the sensory test protocol, each panellist was served with three blank samples (which were purchased from a supermarket) at the beginning of each session, followed by 30 experimental samples. Panellists were required to evaluate tenderness, juiciness, flavour and overall liking of the samples on a nine-point hedonic scale (Meilgaard et al., 2007; Wichchukit and O'Mahony, 2015) (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, and 1 = dislike extremely).

The loin chop samples designated for sensory evaluation were thawed at 4^0C over 24 h before analysis. The loin chops were cooked on a barbeque Spinifex 4 Burner grill unit using conductive dry-heat on a fry grilling hot plate, with heat control knobs set at the *"High"* position. No cooking oil and other additives were used during cooking. Internal meat temperature was monitored using portable instant-read AcuRite meat thermometers (AcuRite,

Lake Geneva, WI, USA). The loin chops were removed from the cooking surface when the internal core temperature reached 70^0C (medium doneness). After being rested for 3 minutes, the bone, subcutaneous fat and visible connective tissues were removed. The cooked meat was cut into 10 cubes of similar size (approximately 1 cm^3). Each cube was placed on a code-labelled disposable plastic plate and randomly served to panellists. The panellists were offered water and unsalted crackers between samples to neutralize taste buds and minimize cross-sample contamination. In each session, the panellist tasted 30 cubes from 30 different lambs, giving a total of 600 replications in both sessions. Thus, each loin chop sample was evaluated by 10 panellists. The data provide adequate statistical robustness and vigour for analysis, and was consistent with Thompson et al. (2005b) who reported that 10 raw consumer scores per meat sample are sufficiently significant to detect differences in sensory traits between samples.

5.3.7. Statistical analysis

All data were analysed using Statistical Analysis System software version 9.2 (SAS Institute, Cary, NC, USA). Summary statistics including means and standard errors were computed and scrutinised for any erroneous data entry. For FA profile analysis, the data were fitted into a General Linear Model (PROC GLM) with oil supplementation, lamb breed, and their interaction as fixed effects, and total lipid percentages, individual and major group FA percentages and contents as dependent variables. For sensory attributes, a mixed model (PROC MIXED) was used to analyse tenderness, juiciness, flavour and overall liking of meat samples as dependent terms. The fixed effects in the model were oil supplementation, lamb breed and their interaction. The random effects in the model included individual panellists, order of tasting and sessions. Significant differences and mean separations at the $P < 0.05$ threshold were performed using Duncan's multiple range and Tukey's probability pairwise comparison tests.

5.4. Results

5.4.1. Feed ingredients, chemical composition and fatty acid profiles

The ingredients and chemical composition of the experimental pellets and lucerne hay are given in Table 5.1. Wheat was the major carrier ingredient (465 - 551 g/kg) in the pellets. The CP, EE and ME contents, and other chemical composition were relatively similar between the five pellets. Lucerne hay had more CP, NDF, ADF and less EE than the pellets.

The total lipid percentage, FA concentration and 18:3n-3 content of feedstuff are presented in Table 5.2. The extracted lipid percentage in pellets were relatively similar ranging from 4.2% to 4.8%. The prominent FA in pellets were 18:2n-6 and 18:1n-9, while 16:0 and 18:3n-3 accounted for the highest FA percentage in lucerne hay. Control pellets had greater PUFA concentration (48.4%) and n-6/n-3 ratio (11.1) compared to oil supplemented pellets. Oil supplemented pellets had greater 18:3n-3 content than control pellets. Lucerne hay contained high 18:3n-3 content (176.2 mg/100g), and had low n-6/n-3 ratio (0.8). EPA, DHA and DPA were not detected in the pellets and lucerne hay.

Table 5.1. Ingredient and nutrient composition of feeds

Item	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
Ingredients, g/kg						
Wheat	513	537	545	551	465	–
Paddy rice	260	230	210	220	280	–
Lupins	170	151	138	147	148	–
Canola oil (ml/kg)	-	25	50		-	–
Flaxseed oil (ml/kg)	-		-	25	50	–
Salt	10	10	10	10	10	–
Limestone	21	21	21	21	21	–
Sheep premix	1	1	1	1	1	–
Ammonium sulfate	12.6	12.6	12.6	12.6	12.6	–
Acid buffer	6.2	6.2	6.2	6.2	6.2	–
Sodium bicarbonate	6.2	6.2	6.2	6.2	6.2	–

Chemical composition, % dry matter						
Dry matter, (%)	89.8	90.2	87.9	90.5	89.4	89.6
Crude protein	14.7	14.5	14.4	14.5	14.5	17.4
NDF	23.8	23.5	23.9	23.7	23.3	46.5
ADF	9.2	9.3	8.9	9.5	9.0	30.9
NFC[1]	50.5	49.9	47.8	50.5	50.7	27.4
Ether extract	4.5	4.6	4.9	4.7	5.0	2.4
Ash	8.0	7.5	8.2	7.1	6.4	7.2
ME[2], MJ/kgDM	10.8	10.9	11.1	10.8	11.0	9.8

[1]NFC = non-fibrous carbohydrates [NFC = 100 − (CP + NDF + EE + ash)]

[2]ME = metabolisable energy.

5.4.2. Muscle fatty acid profile

A marked decrease ($P < 0.05$; Table 5.3) in the n-6/n-3 ratio was observed in lambs fed flaxseed oil pellets compared to lambs offered control pellets, while the n-6/n-3 ratio of lambs in canola oil treatments did not significantly differ from other treatments ($P > 0.05$). Lambs fed flaxseed oil and 5% canola oil pellets had significantly lower 17:0 ($P < 0.05$) than those offered the control pellets. The inclusion of 5% oil in pellets markedly increased ($P < 0.05$; Table 5.3) EPA and DPA contents, whereas these contents were unaffected by supplementing with 2.5% oil. Oil supplementation also significantly increased ($P < 0.05$) DHA content in experimental lamb LTL muscle. As a consequence, the content of EPA+DHA and total n-3 LC-PUFA content of lambs fed 5% oil enriched pellets was significantly greater ($P < 0.01$) than that of lambs in the control treatment. Oil inclusion did not alter total lipid percentage (intermuscular fat (IMF) percentage), and the PUFA/SFA ratio ($P > 0.05$).

The IMF percentages and FA profiles in the LTL muscle were significantly influenced by breed ($P < 0.05$; Table 5.3). Crossbred lambs had greater ($P < 0.05$) IMF percentage than purebred Merinos. First-cross CxM lambs recorded the highest 14:0 (2.2%), 16:0 (23.6%) and total SFA (45.1%) percentages. However, the percentage of 18:2n-6 (4.8%) was lowest in CxM lambs.

Purebred Merinos had significantly greater ($P < 0.05$) EPA (0.9%), DHA (0.3%) and DPA (0.8%) percentages than CxM lambs (0.4%, 0.1% and 0.5% respectively). Purebred Merinos and WxC lambs had markedly greater ($P < 0.05$) total PUFA, n-3 PUFA, n-6 PUFA percentages than CxM lambs, although these percentages did not significantly differ between MxM and WxC lambs. First-cross CxM lambs had a lower PUFA/SFA ratio in LTL muscle than MxM and WxC lambs. However, the n-6/n-3 ratio was not affected by lamb breed. The EPA and DHA contents of purebred Merinos were significantly greater ($P < 0.05$) than those of crossbred lambs. As a consequence, purebred Merinos had markedly greater ($P < 0.001$) total content of EPA+DHA than crossbred lambs. The contents of DPA and total n-3 LC-PUFA were not influenced by lamb breed. There were no significant interactions between oil supplementation and lamb breed.

5.4.3. Visceral adipose tissue fatty acid profile

The supplementation of 5% flaxseed oil significantly increased ($P < 0.05$; Table 5.4) the percentages of 18:3n-3 (2.6%) and DPA (0.12%) in adipose tissue compared to the Control treatment (1.2% and 0.05% respectively). Furthermore, lambs fed 5% flaxseed pellets had

Table 5.2. Total lipid percentage (g fat/100g tissue mass), fatty acid percentage (g/100g total FA) and alpha linolenic acid (18:3n-3) content (mg/100g feed) of experimental diets

	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
Lipid percentage	4.2	4.3	4.7	4.5	4.8	2.2
FA composition[1]						
14:0	0.2	0.5	0.6	0.2	0.2	0.6
15:0	0.1	0.1	0.1	0.1	0.1	0.4
16:0	18.2	16.9	16.5	19.1	19.8	29.6
17:0	0.1	0.1	0.2	0.1	0.1	0.7
18:2n-6	43.4	28.4	26.7	25.6	24.7	19.1
18:3n-3	3.5	3.6	4.3	4.9	7.2	22.1
18:1n-9	23.9	37.5	38.9	32.3	34.1	2.5
18:0	3.4	4.1	4.1	4.4	5.1	4.7
20:4n-6	ND	ND	ND	ND	ND	ND
20:5n-3	ND	ND	ND	ND	ND	ND
20:3n-6	0.3	0.4	0.4	0.4	0.5	0.4
20:4n-3	0.4	0.5	0.2	0.5	0.6	0.5
20:2n-6	0.1	0.1	0.2	0.1	0.1	0.1
20:0	0.5	0.8	0.7	0.7	0.8	1.5
22:5n-6	ND	ND	ND	ND	ND	ND
22:6n-3	ND	ND	ND	ND	ND	ND
22:5n-3	ND	ND	ND	ND	ND	ND
∑SFA	24.1	23.0	25.0	26.7	28.7	47.3
∑MUFA	27.5	42.6	43.3	36.3	37.4	9.4
∑PUFA	48.4	34.4	31.7	37.0	33.8	43.3
PUFA/SFA	2.0	1.5	1.3	1.4	1.2	0.9
∑n-3 PUFA	3.9	4.1	4.8	5.5	7.9	23.6
∑n-6 PUFA	43.8	28.8	27.4	26.0	25.3	19.4
n-6/n-3	11.1	7.0	5.7	4.7	3.2	0.8
FA content						
18:3n-3	61.1	71.3	79.4	84.1	138.2	176.2

[1]∑SFA: total saturated fatty acid includes: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA: total monounsaturated fatty acid includes: 14:1, 16:1n-9, 16:1n-7, 16:1n-5, 16:1n-13, 17:1n-8(+a17:0), 17:1, 18:1n-9, 18:1n-7, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-9, 24:1n-9; ∑PUFA: total polyunsaturated fatty acid includes 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 22:5n-6, 22:6n-3, 22:5n-3, 22:4n-6, 24:6n-3, 24:5n-3; ∑n-3 PUFA: total omega-3 PUFA includes 18:3n-3, 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3; ∑n-6 PUFA: total omega-6 PUFA includes 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6; ND: not detected.

Table 5.3. Effects of oil supplementation and lamb breed on intermuscular fat (IMF) percentage (g fat/100g tissue mass), fatty acid composition (g/100g total FA) and content (mg/100g wet tissue) of *Longissimus thoracis et lumborum* muscle

	Treatment					Breed[1]			SEM[2]	P value[3]		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		T	B	TxB
IMF percentage	2.9	3.1	3.4	3.2	3.3	2.6^{b}	3.4^{a}	3.5^{a}	0.18	0.36	0.02	0.53
FA composition[4]												
14:0	1.5	1.6	1.8	2.1	1.4	1.4^{b}	2.2^{a}	1.6^{b}	0.12	0.39	0.04	0.57
15:0	0.3	0.3	0.3	0.3	0.3	0.2	0.3	0.3	0.02	0.74	0.12	0.73
16:0	21.7	22.3	21.5	23.7	21.8	21.6^{b}	23.6^{a}	21.6^{b}	0.36	0.34	0.04	0.28
17:0	1.2^{a}	1.1^{ab}	1.0^{b}	1.0^{b}	1.0^{b}	1.1	1.1	1.0	0.03	0.05	0.35	0.39
18:2n-6	6.7	5.7	6.5	5.1	5.9	6.5^{a}	4.8^{b}	6.4^{a}	0.26	0.34	0.02	0.56
18:3n-3	1.5	1.6	1.5	1.5	2.0	1. 7	1.4	1.7	0.10	0.18	0.21	0.65
18:1n-9	33.2	35.8	33.0	34.9	32.3	34.0	34.6	33.0	0.53	0.19	0.54	0.18
18:0	16.5	15.3	16.4	15.7	16.3	15.0	16.2	16.7	0.35	0.79	0.12	0.79
20:4n-6	1.5	1.4	1.7	1.1	1.6	1.6	1.2	1.6	0.10	0.52	0.12	0.76
20:5n-3 (EPA)	0.6	0.6	0.8	0.6	0.8	0.9^{a}	0.4^{b}	0.7^{ab}	0.06	0.61	0.01	0.88
20:3n-6	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.2	0.02	0.86	0.20	0.94
20:4n-3	0.6	0.6	0.7	0.5	0. 7	0.7	0.5	0.7	0.05	0.71	0.12	0.49
20:2n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.33	0.50	0.31
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.00	0.67	0.87	0.07
22:5n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.80	0.92	0.41
22:6n-3 (DHA)	0.1	0.2	0.2	0.2	0.2	0.3^{a}	0.1^{b}	0.2^{ab}	0.02	0.62	0.01	0.85
22:5n-3 (DPA)	0.6	0.6	0.7	0.6	0.7	0.8^{a}	0. 5^{b}	0.7^{ab}	0.05	0.62	0.03	0.89
∑SFA	43.0	42.0	42. 8	44.4	42.4	40.9^{c}	45.1^{a}	43.0^{b}	0.44	0.53	0.01	0.81
∑MUFA	43.4	45.3	43.4	44.7	43.6	44.5	44.6	43.2	0.44	0.54	0.37	0.64
∑PUFA	13.6	12.7	13.8	10.9	14.0	14.6^{a}	10.3^{b}	13.8^{a}	0.60	0.51	0.01	0.75
PUFA/SFA	0.3	0.3	0.3	0.2	0.3	0.4^{a}	0.2^{b}	0.3^{a}	0.02	0.24	0.05	0.13
∑n-3 PUFA	3.4	3.7	3.9	3.3	4.5	4.4^{a}	2.9^{b}	4.0^{a}	0.21	0.33	0.01	0.96
∑n-6 PUFA	8.7	7. 7	8.6	6.5	8.0	8.6^{a}	6.4^{b}	8.5^{a}	0.38	0.40	0.04	0.64
n-6/n-3	2.6^{a}	2.3^{ab}	2.2^{ab}	2.0^{b}	1.8^{b}	2.1	2.2	2.2	0.09	0.01	0.39	0.89
FA content												
18:3n-3	24.9	39.2	36.7	40.8	49.3	33.2	48.0	37.4	3.75	0.37	0.53	0.19
20:4n-6	24.9^{b}	32.1^{ab}	36.8^{a}	29.2^{ab}	33.9^{ab}	29.4	35.6	31.9	1.50	0.02	0.63	0.60
EPA	11.3^{b}	13.1^{ab}	17.0^{a}	14.2^{ab}	17.9^{a}	16.9^{a}	14.0^{b}	13.5^{b}	0.85	0.01	0.03	0.79
22:5n-6	1.1	1.9	1.2	1.9	1.3	1.1	1.9	1.5	0.18	0.47	0.27	0.17
DHA	2.8^{b}	4.5^{a}	5.3^{a}	4.2^{a}	4.9^{a}	5.2^{a}	3.9^{b}	4.1^{b}	0.30	0.01	0.05	0.81
DPA	10.8^{b}	13.4^{ab}	16.3^{a}	13.8^{ab}	15.6^{a}	14.0	14.9	13.5	0.68	0.01	0.95	0.64
EPA+DHA	14.1^{b}	17.6^{ab}	22.3^{a}	18.4^{ab}	22.8^{a}	22.1^{a}	17.0^{b}	17.5^{b}	1.10	0.01	0.04	0.90
EPA+DHA+DPA	24.9^{b}	30.9^{ab}	38.7^{a}	32.2^{ab}	38.4^{a}	36.1	32.8	31.0	1.72	0.01	0.32	0.84

[1]CxM: Corriedale x Merino; MxM: Merino x Merino; WxC: White Suffolk x Corriedale.

[2] SEM: standard error of the mean.

[3]Row means bearing different superscripts within a fixed factor significantly differ ($P<0.05$); T: treatment; B: breed; TxB: treatment x breed

[4]Fatty acid groupings used are as indicated in Table 5.2; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid and DHA: docosahexaenoic acid.

Table 5.4. Variation in total lipid percentage (g fat/100g tissue mass), fatty acid composition (g/100g total FA) and content (mg/100g wet tissue) in visceral adipose tissue as influenced by oil supplementation and lamb breed

	Treatment					Breed[1]			SEM[2]	P value[3]		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		T	B	TxB
Lipid percentage	76.5	78.4	81.5	77.0	79.5	77.8	79.6	78.2	2.64	0.56	0.81	0.27
FA composition[4]												
14:0	2.7	2.6	2.8	2.7	2.4	2.5^{b}	3.1^{a}	2.4^{b}	0.12	0.89	0.03	0.04
15:0	0.7	0.6	0.7	0.6	0.6	0.6	0.6	0.6	0.02	0.58	0.59	0.30
16:0	23.9	22.7	22.3	23.2	23.7	23.3	23.4	22.8	0.26	0.31	0.61	0.03
17:0	1.9	1.5	1.7	1.7	1.5	1.8^{a}	1.7^{ab}	1.4^{b}	0.07	0.19	0.05	0.15
18:2n-6	3.0	4.1	3.6	3.1	3.4	3.4	3.6	3.2	0.17	0.28	0.64	0.80
18:3n-3	1.2^{b}	1.6^{ab}	1.9^{ab}	1.9^{ab}	2.6^{a}	1.9	1.6	2.0	0.10	0.05	0.47	0.26
18:1n-9	23.7	24.3	23.5	23.0	23.0	22.9	24.1	23.5	0.33	0.69	0.31	0.54
18:0	26.4	26.1	23.8	25.4	23.1	24.4	24.9	25.5	0.49	0.11	0.63	0.77
20:4n-6	0.08	0.06	0.06	0.06	0.06	0.07^{a}	0.06^{ab}	0.05^{b}	0.01	0.74	0.03	0.86
20:5n-3 (EPA)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.001	0.76	0.29	0.33
20:3n-6	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.002	0.57	0.61	0.94
20:4n-3	ND	ND	ND	ND	ND	ND	ND	ND				
20:2n-6	0.02	0.07	0.07	0.07	0.05	0.05	0.04	0.07	0.01	0.51	0.51	0.15
20:0	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.2	0.01	0.50	0.87	0.51
22:5n-6	0.08	0.07	0.10	0.10	0.07	0.04^{b}	0.13^{a}	0.08^{ab}	0.01	0.83	0.01	0.52
22:6n-3 (DHA)	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.003	0.22	0.35	0.26
22:5n-3 (DPA)	0.05^{b}	0.09^{ab}	0.09^{ab}	0.10^{ab}	0.12^{a}	0.09	0.09	0.09	0.003	0.04	0.86	0.01
∑SFA	57.5^{a}	55.3^{ab}	52.9^{b}	55.5^{ab}	52.8^{b}	54.6	55.4	54.4	0.68	0.03	0.78	0.64
∑MUFA	37.3	38.2	40.5	37.8	38.0	38.7	38.4	38.1	0.55	0.48	0.89	0.52
∑PUFA	5.2^{b}	6.5^{ab}	6.6^{ab}	6.7^{ab}	9.2^{a}	6.7	6.2	7.5	0.21	0.03	0.55	0.27
PUFA/SFA	0.09^{b}	0.12^{ab}	0.13^{ab}	0.11^{ab}	0.17^{a}	0.12	0.11	0.14	0.01	0.04	0.37	0.11
∑n-3 PUFA	1.3^{b}	1.7^{ab}	2.0^{ab}	2.0^{ab}	2.7^{a}	2.0	1.6	2.1	0.06	0.05	0.55	0.21
∑n-6 PUFA	3.1	4.3	3.9	3.4	3.6	3.6	3.9	3.5	0.17	0.25	0.73	0.81
n-6/n-3	2.4^{a}	2.5^{ab}	2.0^{ab}	1.7^{ab}	1.4^{b}	1.8	2.4	1.7	0.38	0.03	0.53	0.98
FA content												
18:3n-3	338.6^{b}	504.6^{ab}	601.7^{ab}	632.7^{ab}	696.1^{a}	570.9	493.0	591.7	55.42	0.02	0.77	0.06
20:4n-6	32.6	25.4	27.5	25.5	27.1	32.1^{a}	30.7^{ab}	19.8^{b}	2.08	0.13	0.05	0.86
EPA	5.7	5.1	5.8	5.9	5.8	5.0	5.8	6.2	0.23	0.54	0.47	0.39
22:5n-6	26.0	28.9	35.5	34.6	25.0	12.5^{b}	48.7^{a}	28.6^{b}	2.94	0.83	0.01	0.04
DHA	4.2	4.8	4.5	5.4	4.3	4.3	4.7	4.9	0.23	0.41	0.26	0.82
DPA	16.4^{b}	24.6^{ab}	27.2^{ab}	33.9^{ab}	38.1^{a}	29.6	27.9	26.6	2.44	0.05	0.61	0.03
EPA+DHA	9.9	9.9	10.4	11.3	10.1	9.3	10.4	11.0	0.33	0.33	0.78	0.57
EPA+DHA+DPA	26.3^{b}	34.5^{ab}	37.6^{ab}	45.2^{ab}	48.2^{a}	38.9	38.3	37.6	2.71	0.04	0.69	0.02

[1]CxM: Corriedale x Merino; MxM: Merino x Merino; WxC: White Suffolk x Corriedale.

[2] SEM: Standard error of the mean.

[3]Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$); T: treatment; B: breed; TxB: treatment x breed; ND: not detected.

[4]Fatty acid groupings used are as indicated in Table 5.2; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid and DHA: docosahexaenoic acid.

significant greater total PUFA (9.2%) and n-3 PUFA (2.7%) concentration than those offered control pellets (5.2% and 1.3% respectively). Lambs from the 5% oil treatments had less total SFA percentage than those from the control treatment. The inclusion of 5% flaxseed oil in lamb diet significantly increased the PUFA/SFA ratio, and reduced the n-6/n-3 ratio in comparison with the control treatment. The 18:3n-3 content in visceral adipose tissue of lambs fed pellets containing 5% flaxseed oil (696.1 mg/100g wet tissue) was markedly greater than that of the control (338.6 mg/100g wet tissue). The inclusion of 5% flaxseed oil also recorded the highest DPA and total n-3 LC-PUFA contents. The total lipid percentage in visceral fat was not influenced by oil supplementation. The proportions of 14:0 and 17:0 in visceral adipose tissue were significantly influenced by lamb breed ($P < 0.05$) (Table 5.4). First-cross CxM lambs had a significantly greater ($P < 0.05$) 14:0 proportion (3.1%) than MxM (2.5%) and WxC lambs (2.4%). The 17:0 percentage of WxC lambs (1.4%) was significantly less than that of purebred Merinos (1.8%). Purebred Merinos recorded the highest 20:4n-6 percentage (0.07%) and content (32.1 mg/100g wet tissue). The percentage and content of 22:5n-6 from CxM lambs was highest. The PUFA/SFA and n-6/n-3 ratios and the n-3 PUFA contents were not affected by breed.

Significant interactions between oil addition and lamb breed on percentages and contents of individual FA were observed. First-cross CxM lambs recorded the highest 14:0 percentage when they were feed pellets containing 2.5% flaxseed oil, while the lowest 14:0 percentage was observed in WxC lambs fed the 5% flaxseed oil pellets. The adipose 16:0 percentage of CxM lambs fed 2.5% canola oil pellets was lowest ($P < 0.05$), while the highest 16:0 percentage

was obtained in MxM lambs from 2.5% canola oil treatment. First-cross CxM lambs offered 5% flaxseed oil pellets had greater DPA percentage and content than WxC lambs fed the control pellets. First-cross CxM lambs fed canola oil pellets had significant greater 22:5n-6 contents than MxM lambs offered pellets containing flaxseed oil.

5.4.4. Meat sensory characteristics

Table 5.5. The effects of omega-3 supplementation and breed on loin lamb chop sensory characteristics

		Tenderness	Juiciness	Flavour	Overall liking
Treatment	Control	7.1	7.2	7.4	7.2
	2.5% canola	7.3	7.4	7.5	7.4
	5% canola	7.0	7.3	7.2	7.2
	2.5% flaxseed	7.2	7.1	7.3	7.2
	5% flaxseed	6.9	7.2	7.4	7.0
Breed[1]	MxM	6.9[b]	6.9[b]	7.2	7.0[b]
	CxM	7.2[a]	7.4[a]	7.4	7.2[ab]
	WxC	7.2[a]	7.3[a]	7.5	7.4[a]
SEM[2]		0.07	0.06	0.06	0.06
P value[3]	Treatment	0.60	0.17	0.79	0.48
	Breed	0.03	0.01	0.10	0.05
	TxB	0.08	0.03	0.34	0.47

[1]CxM: Corriedale x Merino; MxM: Merino x Merino; WxC: White Suffolk x Corriedale.

[2] SEM: standard error of the mean.

[3]Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$); TxB: treatment x breed.

Sensory scores of loin chops were affected by lamb breed, although not flavour (Table 5.5). Purebred Merinos had lower sensory scores ($P < 0.05$) for tenderness and juiciness than crossbred lambs. Furthermore, meat of WxC lambs was assessed with a more overall liking value in comparison with purebred Merinos. Eating quality was not effected by oil supplementation ($P > 0.05$). However, there was a significant interaction between dietary oil inclusion and lamb breed on juiciness, with MxM lambs fed pellets containing 5% oil having the lowest scores (Figure 5.1).

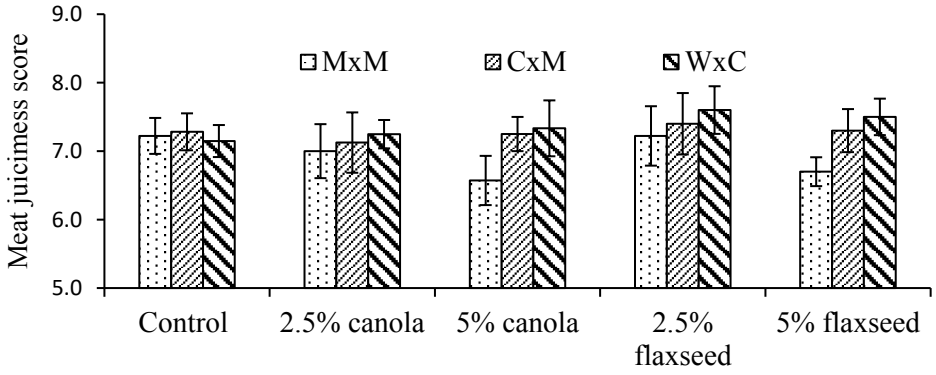

Figure 5.1. Interaction effects on meat juiciness (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale)

5.5. Discussion

5.5.1. The fatty acid profiles of experimental feeds

The differences in FA profiles of experimental pellets resulted from the FA composition of supplemented oils. The most abundant FA in canola and flaxseed oils were oleic acid (18:1n-9; 60% of total FA) and alpha-linoleic (18:3n-3; 55% of total FA) respectively, while 18:3n-3 accounted for approximately 10% of total FA in canola oil. In contrast, LC-PUFA are absent

or account for very minor proportions in these oils (Dubois et al., 2007; Ding et al., 2017). Thus, the inclusion of canola oil or flaxseed oil increased the percentage and content of 18:3n-3 and total n-3 percentage in the pellets. As a consequence, the n-6/n-3 ratio was less in all the pellets. In other studies, canola and/or flaxseed oils have also been used to improve the total n-3 percentage and reduce the n-6/n-3 ratio in animal diets (Jerónimo et al., 2009; Radunz et al., 2009).

5.5.2. Effects of oil supplementation on tissue FA profiles

The IMF percentages of LTL muscle in the present study were greater than the findings of Jerónimo et al. (2009) and Jandasek et al. (2014), but they used younger lambs (3-4 months old) with lighter slaughter weights (30-35 kg). IMF in the LTL muscle and total lipid percentages in visceral adipose tissues were not affected by oil supplementation. This agrees with the findings of Jerónimo et al. (2009) who also did not observe differences in IMF percentages between lambs supplemented with different vegetable oils. They concluded that lipid types added in lamb diets did not influence intramuscular fatty acid content. Furthermore, Dávila-Ramírez et al. (2017) did not find any change in total lipid percentage between lambs fed diets containing 6% soybean oil and non-oil supplemented diets.

Wood et al. (2008) stated that the pattern of fat deposition is a major factor influencing FA profile in ruminant tissues. However, the amount of fat deposited in the tissues depends on animal growth rate and maturity, which in turn, are mainly controlled by diet and animal age (Santos-Silva et al., 2002). The absence of significant oil supplementation effects on total lipid percentage in this study was probably attributable to the relative similarities in chemical composition between experimental diets and the animals used were of the same age.

Table 5.6. Effect of supplementing vegetable oil to lamb on the long-chain polyunsaturated fatty acid percentage of *Longissimus* muscle (g/100 g total fatty acids) and the n-6/n-3 ratio[¥]

Oil source	Duration (week)	EPA	DPA	DHA	n-6/n-3	Reference
Control[1]	5	0.11	0.14	0.05	13.4	Urrutia et al. (2015)
5% flaxseed	5	0.12	0.13	0.02	6.47	Urrutia et al. (2015)
10% flaxseed	5	0.15	0.11	0.03	5.37	Urrutia et al. (2015)
Control[2]	8	0.07	0.20	0.04	5.28	Noci et al. (2011)
6% flaxseed	8	0.12	0.21	0.03	1.75	Noci et al. (2011)
Basal diet[3]	6	0.38	0.70	0.24	6.18	Jerónimo et al. (2010)
6% sunflower and flaxseed (1:2, v/v)	6	0.59	0.73	0.23	2.54	Jerónimo et al. (2010)
Control[4]	6	0.07	0.06	0.31	NA	Radunz et al. (2009)
3% soybean and flaxseed (2:1, v/v)	6	0.04	0.02	0.32	NA	Radunz et al. (2009)
Control[5]	7	0.19	0.46	0.14	7.04	Jerónimo et al. (2009)
6% flaxseed	7	0.50	0.54	0.20	1.60	Jerónimo et al. (2009)

[¥] EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; NA: data not available.

[1]The control diet was mainly composed of soybean meal and barley.

[2]The control diet was based on Megalac (palm-oil based high in 16:0).

[3]The basal diet was composed of manioc and dehydrated lucerne.

[4]The control diet contained mainly ground corn, alfalfa and soybean meal.

[5]The control diet was sunflower based.

It was evident from this study that all diets resulted in an n-6/n-3 ratio below 5/1 in the LTL muscle, which is the ideally recommended value for human diets (WHO/FAO, 1994) . Moreover, the inclusion of flaxseed oil in the pellets caused a significant decrease in the n-6/n-3 ratio in both muscle and visceral adipose tissues. This is due to lower levels of 18:2n-6 and greater levels of 18:3n-3 in the flaxseed oil supplemented diets than in the other diets (Table 5.2). Dubois et al. (2007) and Ding et al. (2017) reported that the 18:3n-3 concentration of flaxseed oil was fivefold greater than that of canola oil. As a consequence, the addition of flaxseed oil to ruminant diets would increase the total muscle n-3 PUFA and potentially the n-3 LC-PUFA content (Woods and Fearon, 2009) and thereby contribute to a decrease in the n-6/n-3 ratio (Noci et al., 2011). A number of feeding trials have investigated the effects of supplementation with flaxseed oil on intramuscular FA composition (Jerónimo et al., 2009; Jerónimo et al., 2010; Noci et al., 2011; Urrutia et al., 2015). In all of these trials, a decreased n-6/n-3 ratio was consistently observed in the LTL muscle of lambs fed flaxseed oil supplemented diets, whereas the n-3 LC-PUFA proportions remained unaffected (Table 5.6). Radunz et al. (2009) also found no differences in n-3 LC-PUFA concentration when lamb finishing diets were supplemented with 3% blend of soybean oil and flaxseed oil (2:1, v/v) into lamb-finishing diets (Table 5.6). Our results for the 2.5% oil supplementation treatments were in agreement with the aforementioned studies.

There were significant increases in muscle n-3 LC-PUFA contents when lambs were supplemented with 5% oil. The oil supplementation effects on meat n-3 LC-PUFA content might have been due to the greater amount of FA intake (Jerónimo et al., 2010). Several *in*

vitro studies confirmed that biohydrogenation of n-3 LC-PUFA in ruminants is limited, even without rumen protection technologies, and its extent depends on the amount of these FA available to ruminal microbes (Dohme et al., 2003; Fievez et al., 2003). Moreover, Cooper et al. (2004) suggested that FA contents reflect their dietary levels. In contrast, other studies conducted in sheep concluded that the profile of absorbed FA is independent of dietary FA due to the high level of ruminal lipid biohydrogenation (Sinclair et al., 2005; Bessa et al., 2007). However, the increased intake and associated update of dietary FA could be indicative that part of the dietary-derived n-3 PUFA intake had escaped biohydrogenation in the rumen (Sinclair et al., 2005). Additionally, Doreau and Ferlay (1994) stated that if large amounts of FA are available in the rumen, it is possible for significant uptake of dietary FA to occur.

An alternative explanation for our results is the further biosynthesis by desaturation and elongation of 18:3n-3 to n-3 LC-PUFA by ruminal microbes, as microbial n-3 LC-PUFA may account for up to 30% of n-3 LC-PUFA flow in the small intestine (Sinclair, 2007). These FA are directly absorbed in the intestine and then stored in the tissue.

According to Food Standards Australia New Zealand (FSANZ, 2012), food can be labelled as a 'source' of n-3 if it contains at least 30 mg of EPA+DHA per standard serve and can be a 'good source' if it contains no less than 60 mg of EPA+DHA per standard serve. A standard serve of red meat for Australia and New Zealand is reported to be 135 g (Hopkins et al., 2014; Ponnampalam et al., 2014a). Thus, the total content of EPA and DHA in the LTL muscle tissue produced from lamb offered 5% canola oil (22.3 mg/100g wet tissue) and 5% flaxseed oil (22.8

mg/100g wet tissue) achieved 30.2 and 30.8 mg per standard serve respectively (Figure 5.2), and thereby readily reached the 'source' level of n-3, while this claimable level was not attained by muscle produced from lamb fed the control and 2.5% oil supplemented diets.

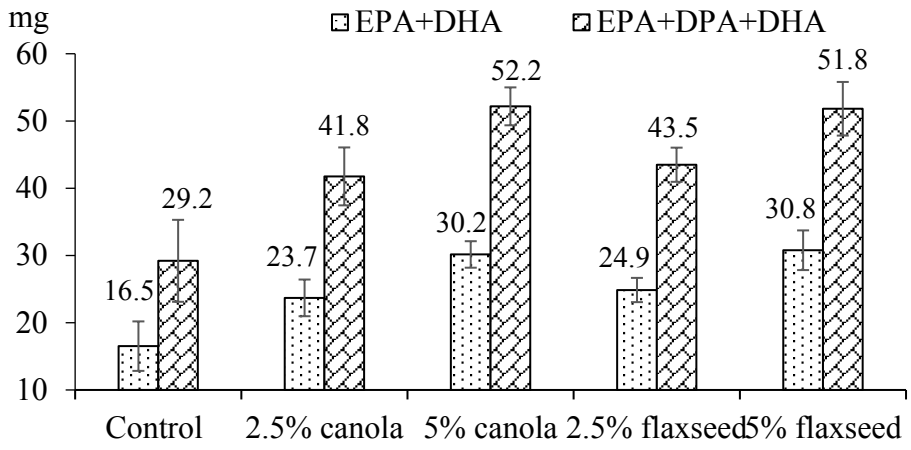

Figure 5.2. The absolute total content of EPA+DHA and EPA+DHA+DPA in *Longissimus thoracis et lumborum* lamb muscle in a per standard serve (135 g) (EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid and DHA: docosahexaenoic acid).

Biochemically, DPA is an intermediary between EPA and DHA in the n-3 synthesis pathway (Kaur et al., 2016) as described in detail in the Literature Review chapter. Many epidemiological studies have demonstrated that DPA consumption is positively correlated with lower platelet aggregation, lower incidence of coronary heart diseases (Phang et al., 2009; Mozaffarian et al., 2013), and improvement in lipid metabolism, and inhibition of inflammation (Chen et al., 2012). Furthermore, Lim et al. (2013) stated that DPA improves mental health. However, the roles of DPA in human health have been largely ignored, perhaps due to its

structural similarity and also it being a negligible component of commercial products such as fish oils compared to the other two main n-3 LC-PUFA – EPA and DHA (NHMRC, 2005; Byelashov et al., 2015). Docosapentaenoic acid contributes approximately 30% of total n-3 LC-PUFA in our diet (Howe et al., 2006). It can serve as a reservoir for EPA and DHA because it is either retro-converted to EPA or elongated to DHA (Miller et al., 2013; Howes et al., 2015). Thus, some reports have suggested that DPA should be included in LC-PUFA intake (Howe et al., 2006; Mozaffarian and Wu, 2012). Indeed, Australia and New Zealand have offered guidelines for DPA intake along with EPA and DHA (NHMRC, 2005). Including DPA in n-3 LC-PUFA intake would boost the total n-3 LC-PUFA content produced in lamb meat to higher values (Clayton, 2014) which is consistent with our results (Figure 5.2).

The levels of n-3 LC-PUFA were very low in the visceral adipose tissue of lambs receiving all experimental diets. Similar findings were observed by Bolte et al. (2002) who investigated the effects of oilseed supplementation on lamb kidney and pelvic fat. Meale et al. (2015) also showed that the levels of EPA, DHA and DPA in both subcutaneous and prerenal adipose tissues of lambs fed various lipid sources were negligible. This likely occurred as a result of a general predisposition of ruminants to preferentially deposit fatty acids in their tissues. Wood et al. (2008) stated that a significant percentage (> 90%) in adipose tissues is triacylglycerol, which has shorter chain and more saturated FA, while the majority of lipid in muscle is phospholipid, which contains a higher portion of long-chain FA and much higher levels of PUFA.

5.5.3. Lamb breed effects on tissue FA profiles

Breed is a major source of variation in tissue FA profile (Fisher et al., 2000; Demirel et al., 2004). The significant impacts of bovine breeds on tissue FA composition have also been reported (Malau-Aduli et al., 1998; Malau-Aduli et al., 2000). The variations might have stemmed from differences in body fatness and cellularity of fat depots during growth (Demirel et al., 2004). De Smet et al. (2004) and Sinclair (2007) stated that the impact of genetic factors on FA composition is markedly less than that of nutritional factors and they are difficult to interpret clearly. Therefore, although genetic effects deserve attention, future emphasis on feeds and feeding strategies is required for further understanding of the key nutritional aspects and factors influencing them in terms of diet and environment.

5.5.4. Effects on sensory quality

In previous studies, the significant increases in the muscle n-3 LC-PUFA content of lamb supplemented with vegetable oils rich in PUFA had no effect on meat sensory quality (Jerónimo et al., 2012; Dávila-Ramírez et al., 2017), which is in agreement with our findings. Other studies conducted in goats and cattle also did not show any significant difference in sensory characteristics of meat when including vegetable oils in the animal diets (Najafi et al., 2012; Castro et al., 2016). In contrast, Francisco et al. (2015) reported that meat obtained from lambs offered a diet supplemented with 8% flaxseed and soybean oil blend (2:1, v/v) had less juiciness and overall liking scores, and a greater score for off-flavour in comparison with meat obtained from lambs fed with 4% oil blend and non-supplemented diets. Moreover, Jaworska

et al. (2016) and Nute et al. (2007) also studied lamb meat quality when offering different oil sources. Their results showed that fish oil enriched diets had detrimental effects on sensory traits compared to other diets containing canola oil or flaxseed oil. They concluded that supplementing with oil enriched in animal diets can alter oxidative degradation and the type of volatiles released during storing and processing, and that these outcomes are associated with the reduction of sensory properties in meat.

The effects of different breeds on sensory quality are significant, although remain contradictory in current literature. Pannier et al. (2014a) showed that sensory scores were greater in Merino lambs compared to first-cross Merino and first-cross terminal lambs. In contrast, Hopkins et al. (2005) found that Merinos generally had lower sensory scores than first-cross terminal lambs, which is consistent with our findings. This could partly be attributed to the greater IMF percentage of crossbred lambs compared to purebred Merinos. Jandasek et al. (2014) and Komprda et al. (2012) reported that increased IMF level resulted in greater scores for sensory properties. However, the mechanisms explaining the impacts on lamb meat sensory characteristics are complicated and still not fully understood (Jaworska et al., 2016). The differences in meat eating quality are affected by a variety of factors associated with livestock attributes such as muscle FA profile, IMF content, growth performance and maturity, which in turn, are influenced by genetics (Hopkins, 2016) and feeding regime (Meale et al., 2015).

5.6. Conclusions

The inclusion of 5% flaxseed oil in the pellets significantly decreased the n-6/n-3 ratio in both muscle and visceral adipose tissues. Lambs offered 5% oil supplementation had greater EPA+DHA and total n-3 LC-PUFA contents in the LTL muscle compared with lambs fed

control pellets. Furthermore, a standard serve (135 g) of meat produced from lambs supplemented with 5% oil in this study contained more than 30 mg of EPA+DHA and reached the claimable 'source' level of n-3 LC-PUFA. With the inclusion of DPA, the total n-3 LC-PUFA content of all experimental lambs would be a 'source' of n-3. The n-3 LC-PUFA contributed very negligible components in the visceral adipose FA profile. Oil supplementation did not cause any significant impact on total lipid percentages and sensory traits. Lamb breed influenced the IMF percentage of LTL muscle and FA profile in both tissues. It also significantly affected meat tenderness, juiciness and overall liking values. Significant interactions between oil supplementation and lamb breed on some visceral adipose FA and meat juiciness score were detected. These findings support the tested hypotheses. In conclusion, canola and flaxseed oils can be effectively used as dietary lipid sources in feedlot regimes in the Australian prime lamb industry. Purebred Merinos produce loin chops with greater EPA+DHA content, but lesser tenderness and overall liking values compared to crossbred lambs. It is proposed that supplementing 5% canola oil or 5% flaxseed oil into the diets of Australian prime lambs could considerably improve the health benefitting n-3 LC-PUFA content in their meat without detrimental impacts on sensory attributes of meat eating quality.

Chapter 6: Omega–3 long-chain fatty acids in the heart, kidney, liver and plasma metabolite profiles of Australian prime lambs supplemented with pelleted canola and flaxseed oils

6.1. Abstract

The objective of the study was to ascertain whether human health beneficial omega–3 long-chain (≥ C_{20}) polyunsaturated fatty acid (n-3 LC-PUFA) content in heart, kidney and liver can be enhanced by supplementing prime lambs with graded levels of canola and flaxseed oil. Health status of the lambs, as a consequence of the supplementation, was also investigated by examining their plasma metabolites. Sixty purebred and first-cross lambs were allocated to one of five treatments of lucerne hay basal diet supplemented with isocaloric and isonitrogenous wheat-based pellets without oil inclusion (Control), or graded levels of canola oil at 2.5% (2.5C), 5% (5C), flaxseed oil at 2.5% (2.5F) and 5% (5F) in a completely randomised design. Pre-slaughter blood, post-slaughter kidney, liver and heart samples were analysed for plasma metabolite and fatty acid profiles. Summations of docosapentaenoic acid and docosahexaenoic acid, and total n-3 LC-PUFA were enhanced in the liver and kidney of 5F supplemented lambs with a marked decrease in n-6/n-3 ratio and significant breed differences detected. There were generally no deleterious impacts on animal health status. A combination of 5% oil supplementation and lamb genetics is an effective and strategic management tool for enhancing n-3 LC-PUFA contents of heart, kidney and liver without compromising lamb health.

6.2. Introduction

The Australian Guide to Healthy Eating and Australian Dietary Guidelines promote health and wellbeing by providing scientific evidence based dietary advice to reduce the risk of high cholesterol, high blood pressure, obesity, type 2 diabetes, cardiovascular disease and cancers (NHMRC, 2017). Therefore, consumers have become more aware and concerned about the relationship between dietary intake and health as their health consciousness increases. High levels of saturated fatty acids (SFA) and low content of polyunsaturated fatty acids (PUFA) in red meat have been implicated in the increased incidence of chronic diseases, especially cardiovascular diseases, diabetes and cancers (Richi et al., 2015; Ekmekcioglu et al., 2017). Enser et al. (1996) reported that lamb contains a greater fat percentage than beef and pork, and lower levels of PUFA in comparison with pork. Therefore, reducing fat content and modifying the fatty acid profile of lamb edible products have been warranted (Adeyemi et al., 2016a) and received much attention (see reviews by De Brito et al. (2017) and Alvarenga et al. (2015)).

The main way of improving the PUFA content in ruminants is the supplementation of PUFA enriched plant oils in their diets (Bessa et al., 2007; Castro et al., 2016). Canola and flaxseed oils, which contain an abundance of α-linoleic acid (ALA, 18:3n-3) (Gillingham et al., 2011; Ding et al., 2017), have been of recent interest in numerous nutritional trials in order to mainly improve n-3 LC-PUFA contents including eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) in sheep meat (Flakemore et al., 2014; Meale et al., 2015; Urrutia et al., 2015; Francisco et al., 2016). Genetic management of livestock for enhancing n-3 LC-PUFA content through selective breeding provides an alternative to nutritional manipulation and it is a cumulative and long-term approach. Several studies have been undertaken to investigate the impacts of different breeds on fatty acid profiles (Wachira et al., 2002; Demirel et al., 2006; Annett et al., 2011). However, these investigations were only limited to the effect of breed on muscle FA

composition. In many countries, the consumption of non-carcass components, for instance heart, liver and kidney, is very common. Furthermore, offal such as various organs can be a cheap source of proteins, minerals and vitamins (Toldrá et al., 2012; Umaraw et al., 2015) and play an important role in processed product formulations (Umaraw et al., 2015). Liver and kidney are typically used in processed food products, such as pies and which may not necessarily be healthy. Thus, lamb edible products include not only meat, but also these visceral organs. Meat and Livestock Australia can strategically promote and encourage the direct intake and export of such offal organs instead of processed "junk food" if there were science-based empirical data suggesting that these offal organs contained health-claimable levels of n-3 LC–PUFA. The effects of different lipid sources and supplemented levels on altering the fatty acid profiles of liver and kidney in cattle and goat have been documented (Rule et al., 1994; Adeyemi et al., 2016a). Kim et al. (2007) also reported the variations in fatty acid composition in liver of Katadhin Dorper lambs fed 4.0% oil supplements with different n-6/n-3 fatty acid ratio. Nonetheless, studies investigating fatty acid profiles of heart, liver and kidney in prime lambs influenced by both dietary canola and flaxseed oil supplementation and breed are scanty and have not been undertaken under pasture-based production system.

Knowledge of haematological metabolite concentrations is valuable in understanding the individual health status and productivity of lambs (Braun et al., 2010). Hence, quantifying the changes in plasma metabolite concentrations in lambs due to feed supplements and genetics is essential (Hegarty et al., 2006b), especially the plasma metabolite profiles of prime lambs supplemented with graded levels of canola and flaxseed oil. The primary objective of this study was to investigate the effects of graded levels of canola and flaxseed oil supplementation to purebred Merino and first-cross prime lambs on heart, liver and kidney fatty acid profiles, including absolute contents, and the plasma metabolites. The secondary aim was to evaluate the interactions of supplementation level with breed.

6.3. Materials and Methods

6.3.1. Location and animals

This research was conducted at the Cressy Research and Demonstration Station, Cressy, Tasmania, Australia, between June and August 2014. The experimental design and procedures were approved by the University of Tasmania Animal Ethics Committee (Permit No. A13839). The study was also in accordance with the 1993 Tasmania Animal Welfare Act and the 2013 Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Sixty weaned ewe (n = 30) and wether (n = 30) prime lambs (seven months of age, mean 33.4 kg liveweight) were used in this feeding trial. The lambs comprised 20 purebred Merinos (MxM), 20 Corriedale x Merino (CxM) and 20 White Suffolk x Corriedale (WxC) first-cross lambs with equal number of ewe and wether lambs represented in each breed.

6.3.2. Experimental design and diets

A completely randomised experimental design balanced by breed and sex was utilised. Lambs were supplemented daily with one kg of isocaloric and isonitrogenous wheat-based pellets and allocated to one of five treatments of 12 lambs per group: no oil inclusion (Control); 2.5% canola oil (2.5C); 5% canola oil (5C); 2.5% flaxseed oil (2.5F) and 5% flaxseed oil (5F) on dry matter basis for 7 weeks after a three-week adaptation period. Lambs had *ad libitum* access to the basal diet of lucerne hay and clean water. Residual feed leftover was removed and weighed prior to fresh feed being offered to experimental lambs at 0900 hours.

6.3.3. Blood and visceral organ sampling

At the end of the feeding trial, blood samples were collected using jugular venipuncture. The lambs were organised for blood sample collection in the cool hours of the morning. Individual lambs within each treatment group were gently restrained in a relaxed sitting position with one

researcher holding their heads ensuring they were comfortably upright and stable on flat ground to minimise individual animal variation and stress. These were stored in tubes containing heparin, immediately chilled in an esky containing ice and later centrifuged at 3000 rpm for 20 min at 4°C. Plasma sub-samples were taken and stored at −20°C for subsequent laboratory analysis.

After collecting blood samples, the lambs were walked to an adjacent commercial abattoir (100 m) and fasted overnight with water available in lairage. They were slaughtered the next day following Meat Standards Australia regulations. Heart, kidney and liver samples were taken immediately after evisceration. All samples were vacuum-sealed, code-labelled and stored at −20°C until fatty acid analysis.

6.3.4. Feed chemical analysis

Concentrate pellet and lucerne hay samples were collected on days 0, 25 and 49 of the experimental period and kept at −20°C for subsequent analyses. At the end of the experiment, the samples were defrosted; the three replicates for each sampling day were pooled and ground through a 1 mm screen. Samples were dried in triplicates in a fan-forced oven at 65°C to a constant weight to determine dry matter (DM) content. Total Nitrogen (N) was quantified using an elemental analyser (PE2400 Series II; Perkin-Elmer Corp, Waltham, MA, USA), and multiplied by 6.25 to estimate crude protein (CP) content. Ether extract (EE) was determined using an ANKOM fat/oil extractor (ANKOM^{XT15}; ANKOM Technology, Macedon, NY, USA). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) contents were measured using an ANKOM fibre analyser (ANKOM^{220}; ANKOM Technology, Macedon, NY, USA). Ash content was quantified by combusting the samples in a furnace at 550°C for 5 hours. Organic matter (OM) was computed as OM = 100 − Ash. Non-fibrous carbohydrates (NFC) was calculated as NFC = 100 − (CP + NDF + EE + Ash) (Mertens, 2002). Metabolisable energy

(ME) was estimated using a near infrared reflectance spectroscopy method (Garnsworthy and Unal, 2004).

6.3.5. Fatty acid and plasma metabolite analyses

The total lipid extraction and fatty acid (FA) profile analysis of feed and visceral samples were undertaken at the Commonwealth Scientific and Industrial Research Organization (CSIRO), Oceans & Atmosphere, Hobart, Tasmania, Australia. The procedures were outlined in detail by Malau-Aduli et al. (2016). In brief, total lipids in 1 g of samples were solvent extracted using a modified Bligh and Dyer (1959) protocol. CH_2Cl_2:MeOH:Milli-Q H_2O (1:2:0.8 v/v) was used in a single-phase overnight process to extract lipids, followed by phase separation with CH_2Cl_2:saline Milli-Q H_2O (1:1 v/v), and then rotary evaporated at 40°C to obtain total lipids.

Aliquots of the total lipid extracts were methylated in methanol:dichloromethane (DCM):concentrated hydrochloric acid (10:1:1 v/v) for 2 h at 80°C to produce fatty acid methyl esters (FAME). Glass test tubes fitted with Teflon-line screw caps (Brandon Scientific Glassblowing, Margate, TAS, Australia) were cooled and 1 mL of Milli-Q® water added, along with 1.8 mL hexane: DCM (4:1 v/v). Tubes were vortexed and centrifuged at 2000 rpm for 5 min to break phase, with the upper, organic layer removed. This extraction step was repeated twice. The organic layer was reduced under a stream of nitrogen gas. DCM, with 1.5 mL of internal injection standard (19:0 FAME) was added. A 7890B gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA, USA) equipped with an Equity™-1 fused silica capillary column (50 m × 0.32 mm internal diameter and 0.1-µm film thickness) (Supelco, Bellefonte, PA, USA), a flame ionisation detector, a split/splitless injector (Agilent Technologies, Palo Alto, CA, USA), and an Agilent Technologies 7683 B series autosampler (Agilent Technologies, Palo Alto, CA, USA) was used to analyse the FAME samples. Fatty acid peaks were quantified by ChemStation software (Agilent Technologies, Palo Alto, CA,

USA). GC-mass spectrometry (GC/MS) analyses were undertaken of selected samples to confirm FA identities and was performed using a Thermo Scientific 1310 GC coupled with a TSQ triple quadrupole (Thermo-Fisher Scientific, Milan, Italy). Samples were injected using a Tripleplus RSH auto sampler with a non-polar HP-5 Ultra 2 bonded–phase column (50 m x 0.32 mm i.d. x 0.17 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The HP-5 column was of similar polarity to the column used for GC analyses. The initial oven temperature of 45°C was held for 1 min, followed by temperature programming at 30°C per min to 140°C then at 3°C per min to 310°C where it was held for 12 min. Helium was used as the carrier gas. Mass spectrometer operating conditions were: electron impact energy 70 eV; emission current 250 µA, transfer line 310°C; source temperature 240°C; scan rate 0.8 scan/sec and mass range 40-650 Da. Mass spectra were acquired and processed with Thermo Scientific XcaliburTM software (Waltham, MA, USA).

Fatty acid profiles comprised percentage (g/100g of total FA (TFA) or %TFA) and content (mg/100 g of wet tissue). Fatty acid percentages were qualitatively calculated from FA area output: FA% = (area of individual FA) x (100)/(total FA area). Fatty acid content was computed as FA (mg/100 g) = (Total lipid percentage) x 0.916 x ((FA%)/100) x 1000 (Clayton, 2014), with 0.916 as a lipid conversion factor (Anderson et al., 1975) as cited by Clayton (2014).

Plasma sub-samples were analysed for metabolite concentrations at the Animal Health Laboratory of the Tasmanian Department of Primary Industries, Parks, Water and Environment (DPIPWE), Launceston, Tasmania, Australia. Cholesterol, urea, calcium, magnesium, beta-hydroxybutyrate (BHB) and glucose were analysed on a Konelab 20XTi Clinical Chemistry Analyser (Thermo Scientific, Waltham, MA, USA).

6.3.6. Statistical analysis

All data were analysed using Statistical Analysis System (SAS, 2014). Summary statistics including means and standard errors were computed and scrutinised for any erroneous data

entry prior to running an analysis of variance (ANOVA). Since lambs were repeatedly weighed every week to compute average daily gains; feed samples taken on days 0, 25 and 49 of the experimental period and offal analysis was based on three replicates per organ, the need for a repeated measures analysis of variance model was warranted. The data were fitted into a repeated measures General Linear Model (PROC GLM) with oil supplementation, lamb breed, and their interaction as fixed effects, and total lipid percentages, FA profiles and plasma metabolites as dependent variables. Significant differences and mean separations at the $P < 0.05$ threshold were performed using Tukey′s probability pairwise comparison tests.

6.4. Results

6.4.1. Feed ingredients, chemical composition and fatty acid profiles

The ingredients, chemical composition and fatty acid profiles of the experimental pellets and lucerne hay are given in Table 6.1. The major carrier ingredient in the pellets was wheat (465–551 g/kg). The DM, CP, EE and ME contents, and other chemical composition were relatively similar between the five treatment pellets. Lucerne hay had greater CP, NDF, ADF contents, and less EE and ME contents than the pellets.

The prominent unsaturated fatty acids in pellets were 18:2n-6 and 18:1n-9, while 18:2n-6 and 18:3n-3 were the greatest unsaturated fatty acids in lucerne hay. The control pellets had greater PUFA composition (48.4 g/100 g FA), but less PUFA/SFA and n-6/n-3 ratios (2.0 and 11 respectively) compared to the oil supplemented pellets. Lucerne hay had low PUFA/SFA and n-6/n-3 ratios (0.9). Eicosapentaenoic acid, DHA and DPA were not detected in the pellets and lucerne hay.

6.4.2. Heart fatty acid profile

The inclusion of 5% flaxseed oil in pellets significantly reduced the n-6/n-3 ratio and 17:0 percentage ($P < 0.05$; Table 6.2) in comparison with the control pellets. However, oil

supplementation did not result in any significant differences in the other FA concentration, PUFA contents and total lipid content of heart tissues.

Lamb breed significantly affected some FA concentration ($P < 0.05$; Table 6.2). Purebred Merinos had greater 17:0 (1.1 g/100 g FA) and total MUFA (26.7 g/100 g FA) concentration than W×C lambs (0.9 g/100 g FA and 23.8 g/100 g FA respectively). In contrast, the DPA and total n-6 PUFA percentages of W×C lambs were greater than those of M×M. There were no significant differences in PUFA contents and total lipid content of heart between the breeds.

Significant interactions between oil addition and lamb breed on some heart FA composition and PUFA/SFA ratio were detected (Figure 6.1). First-cross W×C lambs recorded the greatest DPA concentration (1.7 g/100 g FA) when they were fed pellets containing 5% flaxseed oil, while the lowest DPA concentration was observed in M×M lambs fed 2.5% canola oil pellets (0.8 g/100g FA) (Figure 6.1a). Furthermore, M×M lambs fed 2.5% canola oil pellets had greater total SFA concentration than crossbred lambs offered the same pellets (Figure 6.1b), and contained less total PUFA than crossbred lambs fed the same pellets and MxM and WxC lambs offered 5% flaxseed oil pellets (Figure 6.1c). Consequently, the PUFA/SFA ratio of MxM lambs fed pellets containing 2.5% canola oil was significantly less than that of crossbred lambs fed the same pellets, and MxM lambs fed 5% flaxseed pellets (Figure 6.1d).

Table 6.1. Ingredients, chemical composition and fatty acid percentage of feeds

Item	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	Lucerne hay
Ingredients (g/kg)						
Wheat	513	537	545	551	465	–
Paddy rice	260	230	210	220	280	–
Lupins	170	151	138	147	148	–
Canola oil (ml/kg)	-	25	50	-	-	–
Flaxseed oil (ml/kg)	-	-	-	25	50	–
Salt	10	10	10	10	10	–
Limestone	21	21	21	21	21	–
Sheep premix	1	1	1	1	1	–
Ammonium sulfate	12.6	12.6	12.6	12.6	12.6	–
Acid buffer	6.2	6.2	6.2	6.2	6.2	–
Sodium bicarbonate	6.2	6.2	6.2	6.2	6.2	–
Chemical composition (% dry matter)[1]						
Dry matter, (%)	89.8	90.2	87.9	90.5	89.4	89.6
Crude protein	14.7	14.5	14.4	14.5	14.5	17.4
NDF	23.8	23.5	23.9	23.7	23.3	46.5
ADF	9.2	9.3	8.9	9.5	9.0	30.9
NFC	50.5	49.9	47.8	50.5	50.7	27.4
Ether extract	3.0	4.6	5.7	4.2	5.1	2.4
Ash	8.0	7.5	8.2	7.1	6.4	7.2
ME (MJ/kgDM)	10.7	10.9	11.1	10.8	11.1	9.8
Fatty acid percentage (g/100g total FA)[2]						
14:0	0.2	0.5	0.6	0.2	0.2	0.6
15:0	0.1	0.1	0.1	0.1	0.1	0.4
16:0	18.2	16.9	16.5	19.1	19.8	29.6
17:0	0.1	0.1	0.2	0.1	0.1	0.7
18:2n-6	43.4	28.4	26.7	25.6	24.7	19.1
18:3n-3	3.5	3.6	4.3	4.9	7.2	18.8
18:1n-9	23.9	38.9	37.5	32.3	34.1	5.6
18:0	3.4	4.1	4.1	4.4	5.1	4.7
20:3n-6	0.3	0.4	0.4	0.4	0.5	0.4
20:4n-3	0.4	0.5	0.2	0.5	0.6	0.5
20:2n-6	0.1	0.1	0.2	0.1	0.1	0.1
20:0	0.5	0.8	0.7	0.7	0.8	1.5
∑SFA	24.1	23.0	25.0	26.7	28.7	47.3
∑MUFA	27.5	42.6	43.3	36.3	37.5	12.7
∑PUFA	48.4	34.4	31.7	37.0	33.8	40.0
PUFA/SFA	2.0	1.5	1.3	1.4	1.2	0.9
∑n-3 PUFA	3.9	4.1	4.8	5.5	7.9	20.5
∑n-6 PUFA	43.8	28.8	27.4	26	25.3	19.4
n-6/n-3	11.1	7.0	5.7	4.7	3.2	0.9

[1]NFC: non-fibrous carbohydrates [NFC = 100 − (CP + NDF + EE + ash)]; ME: metabolisable energy.
[2]∑SFA: total saturated fatty acid includes: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0; ∑MUFA: total monounsaturated fatty acid includes: 14:1, 16:1n-9, 16:1n-7, 16:1n-5, 16:1n-13, 17:1n-8 + a17:0, 17:1, 18:1n-9, 18:1n-7, 18:1, 19:1, 20:1n-11, 20:1n-9, 20:1n-7, 20:1n-5, 22:1n-9, 22:1n-11, 22:1n-9, 24:1n-9; ∑PUFA: total polyunsaturated fatty acid includes: 18:3n-6, 18:2n-6, 18:3n-3, 20:4n-3, 20:4n-6, 20:5n-3, 20:3n-6, 20:2n-6, 22:5n-6, 22:6n-3, 22:5n-3, 22:4n-6, 24:6n-3, 24:5n-3; ∑n-3 PUFA: total omega–3 PUFA includes 18:3n-3, 20:5n-3, 20:4n-3, 22:6n-3, 22:5n-3; ∑n-6 PUFA: total omega–6 PUFA includes 18:3n-6, 18:2n-6, 20:4n-6, 20:3n-6, 20:2n-6, 22:5n-6, 22:4n-6.

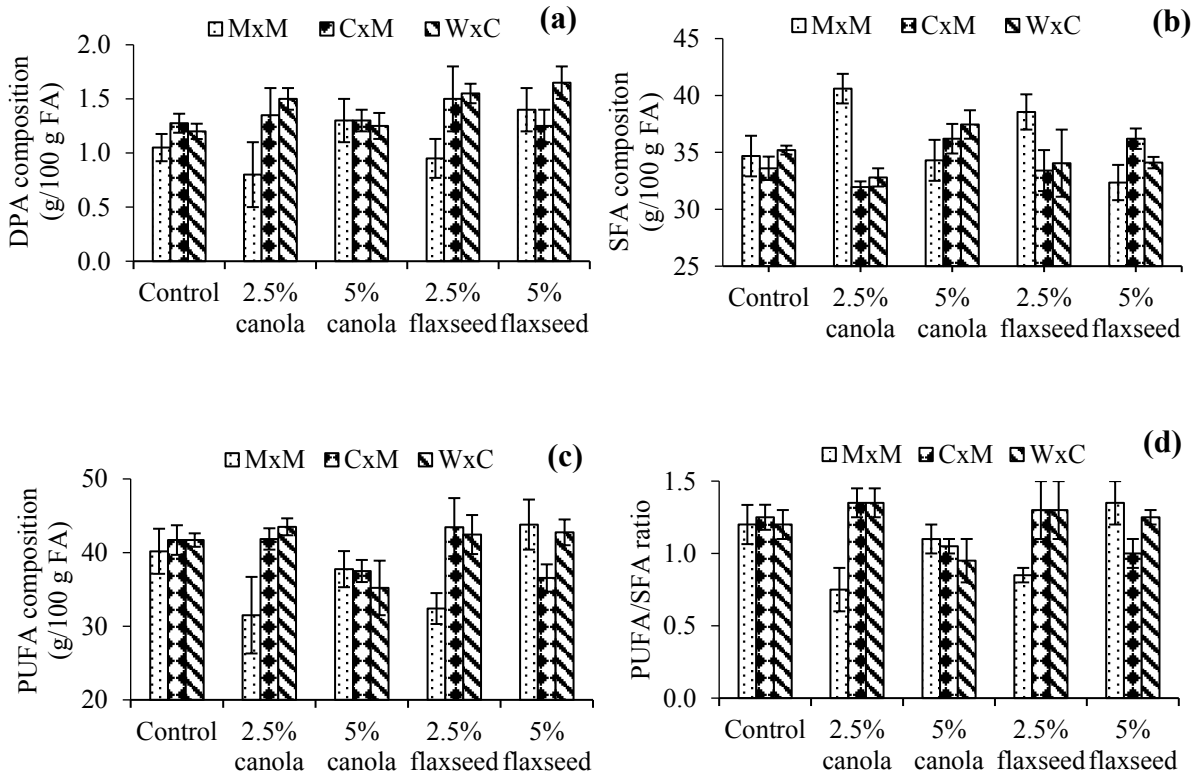

Figure 6.1. Interaction effects on: (a) docosapentaenoic acid, (b) total SFA, (c) total PUFA concentration (g/100 g FA) and (d) PUFA/SFA ratio of lamb hearts (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale)

Table 6.2. Effects of omega–3 oil supplementation and breed on heart fatty acid profile of prime lambs

Item[1]	Control	Treatment				Breed[2]			SEM[3]	P value[4]		
		2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		T	B	T×B
Total lipid (g fat/100g wet tissue)	2.6	2.7	2.8	2.9	2.4	2.8	2.7	2.5	0.09	0.35	0.23	0.16
Percentage (g/100g total FA)												
14:0	0.6	0.6	0.8	0.6	0.5	0.6	0.7	0.5	0.05	0.68	0.33	0.24
15:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.36	0.94	0.30
16:0	12.9	12.5	13.1	12.9	12.7	12.9	12.9	12.7	0.22	0.93	0.63	0.06
17:0	1.1[a]	1.0[ab]	1.0[ab]	0.9[ab]	0.8[b]	1.1[a]	1.0[ab]	0.9[b]	0.03	0.04	0.02	0.07
18:2n-6	23.8	21.0	21.0	21.9	22.4	20.9	23.1	22.9	0.48	0.11	0.06	0.07
18:3n-3	2.0	1.8	2.5	2.1	2.7	2.5	2.1	1.7	0.18	0.53	0.24	0.56
18:1n-9	13.4	15.5	15.6	14.2	12.8	14.9	14.7	12.9	0.45	0.17	0.08	0.39
18:0	17.2	18.4	18.4	17.9	17.5	18.3	17.3	17.6	0.27	0.35	0.12	0.06
20:4n-6 (ARA)	5.6	6.2	4.3	5.8	5.2	5.1	4.8	6.5	0.33	0.33	0.06	0.06
20:5n-3 (EPA)	1.2	1.3	1.2	1.3	1.4	1.2	1.3	1.3	0.04	0.35	0.33	0.07
20:3n-6	0.5	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.01	0.22	0.37	0.44
20:4n-3	1.4	1.4	1.5	1.6	1.7	1.4	1.5	1.6	0.06	0.65	0.47	0.49
20:2n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.81	0.77	0.91
20:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.99	0.74	0.87
22:5n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.25	0.23	0.23
22:6n-3 (DHA)	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.7	0.03	0.85	0.56	0.72
22:4n-6	0.2	0.2	0.1	0.2	0.2	0.1	0.1	0.2	0.01	0.72	0.20	0.57
22:5n-3 (DPA)	1.1	1.1	1.2	1.3	1.4	1.2[b]	1.3[ab]	1.4[a]	0.05	0.19	0.04	0.02
22:0	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.3	0.01	0.74	0.22	0.83
23:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.37	0.56	0.74
24:0	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.85	0.46	0.67
∑SFA	34.5	35.1	35.9	35.2	34.2	35.5	34.5	34.8	0.46	0.63	0.31	0.01
∑MUFA	24.3	26.3	27.3	25.3	24.8	26.7[a]	25.5[ab]	23.8[b]	0.50	0.27	0.04	0.30

∑PUFA	41.2	38.6	36.8	39.5	41.0	37.8	40.0	41.4	0.87	0.30	0.08	0.03
PUFA/SFA	1.2	1.2	1.0	1.2	1.2	1.1	1.2	1.2	0.04	0.52	0.06	0.01
∑n-3 PUFA	6.5	6.4	6.9	6.6	7.9	7.0	6.7	6.7	0.25	0.36	0.90	0.26
∑n-6 PUFA	30.3	28.1	26.1	28.6	28.5	26.9^{b}	28.7^{ab}	30.3^{a}	0.68	0.17	0.02	0.06
n-6/n-3	4.8^{a}	4.4^{ab}	4.1^{ab}	4.3^{ab}	3.6^{b}	4.0	4.4	4.6	0.15	0.02	0.28	0.83
Content (mg/100 g wet tissue)												
18:3n-3	24.1	26.7	24.6	27.5	31.0	31.7	18.7	26.7	2.19	0.83	0.09	0.81
20:4n-6	77.1	101.7	59.9	68.8	71.0	68.6	60.4	99.0	7.44	0.55	0.08	0.58
EPA	13.7	15.7	15.4	18	20.5	16.1	13.1	20.3	1.37	0.64	0.07	0.56
22:5n-6	1.1	1.1	1.2	1.1	0.9	1.0	1.0	1.3	0.09	0.89	0.23	0.94
DHA	7.8	8.0	8.1	9.3	10.4	9.2	6.4	10.1	0.71	0.79	0.14	0.90
DPA	14.0	15.3	16.1	18.0	20.0	15.3	13.4	20.6	1.37	0.72	0.07	0.58
EPA + DHA	21.5	23.7	23.5	27.3	30.9	25.3	19.5	30.4	2.02	0.67	0.09	0.72
EPA + DHA + DPA	35.5	39.0	39.6	45.3	50.9	40.6	32.9	51.0	3.36	0.69	0.08	0.66

[1]All definitions and abbreviations are as indicated in Table 6.1; FA: fatty acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid and DHA: docosahexaenoic acid.

[2]MxM: purebred Merino; CxM: Corriedale and Merino crossbred; WxC: White Suffolk and Corriedale crossbred;

[3] SEM: standard error of the mean;

[4] T: treatment; B: breed; TxB: treatment x breed. Row means bearing different superscripts within a fixed factor differ significantly ($P < 0.05$). In all lamb tissues (Tables 6.2-6.4), low levels of trans 18:1 isomers and conjugated linoleic acid (CLA) isomers were also present.

6.4.3. Liver Fatty Acid Profile

The lambs fed pellets containing 5% flaxseed oil had significantly greater liver DHA (3.4 g/100 g FA) and total n-3 PUFA (11.5 g/100 g FA) percentages ($P < 0.05$; Table 6.3) than the lambs offered the control pellets (2.4 g/100 g FA and 9.2 g/100 g FA respectively). The inclusion of 5% flaxseed oil in pellets also reduced 16:0 and 17:0 concentration, and n-6/n-3 ratio in lamb livers compared to the control pellets. It was apparent that the livers contained high levels of EPA + DHA (148.1–206.9 mg/100 g wet tissue) and total n-3 LC-PUFA (270.5–379.8 mg/100g wet tissue). However, these contents were not influenced by oil supplementation. Lamb breed did not affect the total lipid percentage and FA profile of liver. There were no significant interactions between oil inclusion and lamb breed on liver total lipid percentage and FA profiles.

6.4.4. Kidney Fatty Acid Profile

Supplementing flaxseed oil in the pellets resulted in increases in the EPA and DPA concentration of lamb kidney compared to the control pellets ($P < 0.05$; Table 6.4). Furthermore, the kidneys of lambs fed pellets containing flaxseed oil were greater in total n-3 PUFA concentration than those of lambs from the control and 2.5% canola oil groups. The n-6/n-3 ratio in kidneys significantly reduced for lambs offered flaxseed oil pellets. Oil supplementation significantly reduced the 17:0 concentration of lamb kidney. The incorporation of both 5% canola and 5% flaxseed oils in the pellets reduced 22:5n-6 content, while there were not significant differences in other PUFA contents including EPA + DHA and total n-3 LC-PUFA.

It was observed that lamb breed influenced variations in some kidney fatty acids ($P < 0.05$). First-cross WxC lambs recorded the greatest 20:4n-6 (14.2 g/100 g FA) and DPA (3.2 g/100 g FA) concentration. However, they had less 17:0 and DHA percentages in liver than MxM and CxM lambs. The livers contained more than 60 mg of EPA + DHA and 100 mg of total

n-3 LC-PUFA in 100 g wet tissue (Table 6.4). The DHA and EPA + DHA contents of MxM liver were greater than those of CxM liver. There were no significant differences in kidney total lipid percentage between the breeds. Significant interactions between oil inclusion and lamb breed on the total lipid percentage and FA profiles of kidney were not observed. Liver and kidney of prime lambs had high contents of EPA + DHA and total n-3 LC-PUFA. These visceral organs can be classified as good sources of n–3. A marked decrease in n-6/n-3 ratio of the investigated organs was observed when 5% flaxseed oil was added to the diet of finishing lambs. Supplementing 5% flaxseed oil also increased the DHA and total n-3 PUFA concentration of liver. Addition of flaxseed oil resulted in increases in the EPA, DPA and total n-3 PUFA concentration of kidney. First-cross WxC lambs had greater DPA concentration of heart and kidney than MxM. Lamb breed affected the DHA and EPA + DHA contents of kidney and plasma glucose concentration. Significant interactions between oil addition and lamb breed on heart FA composition were observed.

6.4.5. Plasma Metabolites

Plasma metabolite concentrations of the experimental lambs are presented in Table 6.5. The glucose concentration of WxC lambs (4.5 mmol/L) was significantly greater ($P < 0.05$) than that of MxM lambs (4.0 mmol/L). Moreover, there was a significant interaction between oil supplementation and lamb breed on glucose concentration. First-cross WxC lambs fed pellets containing 2.5% canola oil had greater glucose concentration than MxM lambs fed the control and 2.5% canola oil pellets (Figure 6.2). Oil supplementation did not cause significant alteration in lamb plasma metabolites. However, the lamb urea concentration exceeded its normal ranges. No significant differences in plasma metabolite profiles between supplemented and control lambs imply that there were no obvious health disadvantages to the lambs as a result of oil inclusion.

Table 6.3. Liver fatty acid profile of prime lambs as influenced by omega–3 oil supplementation and breed

Item[1]	Control	Treatment				Breed			SEM	P Value		
		2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		T	B	TxB
Total lipid (g fat/100g wet tissue)	6.3	6.6	6.2	6.3	6.4	6.4	6.6	6.0	0.16	0.99	0.37	0.74
Percentage (g/100g total FA)												
14:0	0.5	0.4	0.4	0.4	0.4	0.4	0.5	0.4	0.02	0.44	0.22	0.22
15:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.22	0.12	0.59
16:0	18.0^{a}	17.4^{ab}	17.4^{ab}	17.3^{ab}	15.9^{b}	17.2	17.3	17.2	0.28	0.03	0.85	0.47
17:0	1.4^{a}	1.3^{ab}	1.3^{ab}	1.3^{ab}	1.2^{b}	1.4	1.3	1.3	0.03	0.02	0.24	0.56
18:2n-6	10.5	10.0	10.1	9.7	10.0	10.5	9.7	10.1	0.20	0.68	0.35	0.59
18:3n-3	2.0	2.3	2.5	2.5	2.7	2.4	2.5	2.1	0.10	0.17	0.53	0.66
18:1n-9	20.7	20.5	20.7	18.1	18.8	19.9	20.2	19.6	0.44	0.30	0.88	0.61
18:0	18.9	19.6	19.3	21.7	20.4	19.4	19.9	20.2	0.41	0.17	0.70	0.36
20:4n-6 (ARA)	4.2	4.6	3.1	4.4	4.5	3.7	4.7	4.0	0.33	0.73	0.57	0.77
20:5n-3 (EPA)	1.3	1.4	1.3	1.3	1.6	1.3	1.5	1.3	0.06	0.68	0.51	0.27
20:3n-6	0.7	0.7	0.7	0.6	0.6	0.7	0.7	0.7	0.03	0.50	0.86	0.07
20:4n-3	0.2	0.1	0.2	0.2	0.1	0.2	0.2	0.2	0.01	0.15	0.47	0.82
20:2n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.27	0.30	0.54
20:0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1				
22:5n-6	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.33	0.26	0.70
22:6n-3 (DHA)	2.4^{b}	2.5^{b}	2.6^{ab}	2.9 ^{ab}	3.4^{a}	2.9	2.6	2.5	0.13	0.03	0.50	0.18
22:4n-6	0.5	0.4	0.4	0.4	0.3	0.4	0.4	0.5	0.02	0.26	0.32	0.66
22:5n-3 (DPA)	3.3	3.1	3.4	3.4	3.7	3.1	3.3	3.6	0.12	0.64	0.24	0.47
22:0	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.01	0.62	0.99	0.92
23:0	0.4	0.4	0.4	0.3	0.3	0.4	0.4	0.4	0.01	0.71	0.83	0.21
24:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.80	0.84	0.06
∑SFA	40.8	41.3	41.4	44.1	40.6	41.2	41.6	41.8	0.54	0.23	0.90	0.12
∑MUFA	32.6	32.0	33.1	29.2	31.1	32.1	31.5	31.6	0.54	0.29	0.91	0.68
∑PUFA	26.6	26.7	25.5	26.7	28.3	26.7	26.9	26.6	0.59	0.92	0.98	0.47

PUFA/SFA	0.7	0.6	0.6	0.6	0.7	0.6	0.6	0.6	0.07	0.69	0.74	0.21
∑n-3 PUFA	9.2^{b}	9.6^{ab}	9.7^{ab}	10.3^{ab}	11.5^{a}	9.9	10.0	9.7	0.32	0.04	0.99	0.26
∑n-6 PUFA	16.5	16.3	14.8	15.6	15.9	15.9	16.0	15.9	0.40	0.73	0.96	0.67
n-6/n-3	1.8^{a}	1.7^{ab}	1.6^{ab}	1.6^{ab}	1.4^{b}	1.6	1.7	1.7	0.05	0.03	0.96	0.57
Content (mg/100 g wet tissue)												
18:3n-3	79.3	86.7	115.7	111.7	118.9	107.9	99.6	88.2	7.20	0.36	0.77	0.57
20:4n-6	186.0	176.6	135.6	225.2	202.5	177.0	192.6	184.2	19.89	0.85	0.96	0.88
EPA	50.9	53.5	58.2	64.8	71.6	56.9	61.2	56.0	4.73	0.86	0.90	0.59
22:5n-6	9.7	9.8	9.7	7.4	8.0	10.6	7.8	8.9	0.72	0.77	0.30	0.91
DHA	97.2	99.1	108.8	142.0	146.8	128.7	110.0	106.0	9.23	0.49	0.71	0.75
DPA	131.1	118.0	156.0	167.4	161.4	140.5	138.6	153.4	11.95	0.76	0.77	0.69
EPA + DHA	148.1	152.5	167.0	206.9	218.3	185.6	171.2	162.0	13.53	0.61	0.88	0.72
EPA + DHA + DPA	279.1	270.5	323.0	374.3	379.8	326.1	309.9	315.5	24.89	0.70	0.97	0.74

[1]All definitions and abbreviations are as indicated in Tables 6.1 and 6.2. Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$).

Table 6.4. Variation in kidney fatty acid profile of prime lambs as influenced by omega–3 oil supplementation and breed

Item[1]	Control	Treatment				Breed			SEM	*P* Value		
		2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC		T	B	TxB
Total lipid (g fat/100g wet tissue)	3.0	3.1	2.8	3.0	2.9	3.0	3.0	3.0	0.05	0.53	0.65	0.42
Percentage (g/100g total FA)												
14:0	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.02	0.44	0.77	0.65
15:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.68	0.58	0.68
16:0	17.0	17.2	16.5	16.4	16.0	16.6	17.0	16.3	0.19	0.53	0.47	0.94
17:0	1.2^{a}	1.1^{b}	1.1^{b}	1.0^{bc}	0.9^{c}	1.1^{a}	1.1^{a}	1.0^{b}	0.02	0.00	0.02	0.07
18:2n-6	12.7	12.6	12.5	12.7	12.6	12.6	12.4	12.8	0.17	0.93	0.84	0.90
18:3n-3	1.1	1.1	1.2	1.2	1.5	1.2	1.3	1.1	0.06	0.22	0.49	0.11
18:1n-9	11.9	12.7	11.7	11.3	11.6	12.1	12.4	11.1	0.25	0.52	0.12	0.51
18:0	17.4	17.5	17.9	17.5	17.8	17.7	17.0	18.1	0.18	0.89	0.16	0.96
20:4n-6	13.8	13.7	13.2	12.7	12.1	12.5^{b}	13.1^{ab}	14.2^{a}	0.29	0.27	0.04	0.69
20:5n-3 (EPA)	1.9^{b}	2.0^{b}	2.3^{ab}	2.5^{a}	2.8^{a}	2.3	2.0	2.3	0.09	0.01	0.08	0.80
20:3n-6	0.9	0.8	0.8	0.9	0.8	0.8	0.8	0.9	0.03	0.86	0.11	0.72
20:4n-3	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.02	0.63	0.17	0.71
20:2n-6	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.01	0.91	0.65	0.27
20:0	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.01	0.30	0.29	0.32
22:5n-6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.01	0.65	0.79	0.85
22:6n-3 (DHA)	2.3	2.3	2.4	2.4	2.4	2.5^{a}	2.4^{a}	2.1^{b}	0.07	0.88	0.03	0.11
22:4n-6	0.4^{a}	0.4^{a}	0.3^{ab}	0.3^{ab}	0.2^{b}	0.3	0.4	0.4	0.02	0.04	0.14	0.88
22:5n-3 (DPA)	2.7^{b}	2.8^{ab}	3.1^{ab}	3.2^{a}	3.3^{a}	2.8^{b}	2.9^{b}	3.2^{a}	0.07	0.04	0.05	0.29
22:0	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.2	0.04	0.85	0.33	0.93
23:0	0.4	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.01	0.48	0.09	0.30
24:0	1.4	1.3	1.3	1.2	1.2	1.3	1.2	1.4	0.05	0.77	0.22	0.99
ΣSFA	40.5	40.5	40.0	39.4	39.6	40.4	39.7	40.3	0.26	0.70	0.64	0.94
ΣMUFA	21.1	21.4	20.5	21.7	22.0	21.5	22.6	19.7	0.43	0.88	0.06	0.97

ΣPUFA	38.4	38.1	39.5	38.9	38.4	38.1	37.7	40.0	0.40	0.87	0.10	0.79
PUFA/SFA	0.9	0.9	1.0	1.0	1.0	0.9	0.9	1.0	0.01	0.93	0.45	0.82
Σn-3 PUFA	8.7^{b}	8.8^{b}	9.8^{ab}	10.0^{a}	10.3^{a}	9.5	8.8	9.7	0.19	0.01	0.06	0.70
Σn-6 PUFA	28.2	27.5	28.0	27.1	26.1	26.7	27.1	28.7	0.38	0.49	0.22	0.86
n-6/n-3	3.3^{a}	3.2^{ab}	2.9^{abc}	2.7^{bc}	2.5^{c}	2.8	3.1	3.0	0.08	0.02	0.39	0.92
Content (mg/100 g wet tissue)												
18:3n-3	18.6	19.7	19.9	23.6	24.6	22.0	22.5	18.6	1.72	0.78	0.63	0.18
20:4n-6	243.6	245.0	223.2	204.3	202.7	234.5	207.0	239.6	9.35	0.46	0.66	0.48
EPA	33.7	37.6	38.1	41.7	45.5	43.0	32.3	39.9	2.00	0.39	0.17	0.86
22:5n-6	2.2^{a}	2.2^{a}	1.3^{b}	1.5^{ab}	1.2^{b}	1.8	1.6	1.9	0.13	0.02	0.82	0.67
DHA	39.2	39.2	39.3	40.5	42.1	46.5^{a}	33.0^{b}	40.9^{ab}	1.87	0.98	0.05	0.28
DPA	50.3	49.7	50.6	51.1	51.3	52.7	45.5	53.5	1.93	0.92	0.44	0.35
EPA + DHA	72.9	76.8	77.4	82.2	87.6	89.5^{a}	65.3^{b}	80.8^{ab}	3.45	0.87	0.05	0.70
EPA + DHA + DPA	123.2	126.5	128.0	133.3	138.9	142.2	110.8	134.3	5.14	0.96	0.16	0.56

[1]All definitions and abbreviations are as indicated in Tables 6.1 and 6.2. Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$).

Table 6.5. Influence of omega–3 oil supplementation and breed on plasma metabolite profiles of prime lambs (mmol/L)

Item[1]	Treatment					Breed			SEM	Normal Range	P Value		
	Control	2.5% canola	5% canola	2.5% flaxseed	5% flaxseed	MxM	CxM	WxC			T	B	TxB
Cholesterol	1.3	1.5	1.5	1.4	1.2	1.3	1.4	1.4	0.04	1.1–1.5	0.13	0.62	0.92
Urea	7.8	7.7	7.5	7.6	7.5	7.8	7.4	7.3	0.16	2.8−7.2	0.27	0.44	0.93
Calcium	2.6	2.6	2.7	2.6	2.7	2.7	2.7	2.6	0.02	2.4−3.2	0.26	0.15	0.61
Magnesium	1.1	1.0	1.0	1.0	1.0	1.0	1.0	1.1	0.02	0.8−1.2	0.83	0.64	0.62
BHB	0.3	0.3	0.4	0.3	0.4	0.3	0.4	0.3	0.02	0.0−0.8	0.66	0.85	0.95
Glucose	4.2	4.5	4.3	4.1	4.2	4.0^{b}	4.3^{ab}	4.5^{a}	0.07	2.8−4.5	0.48	0.03	0.04

[1]As indicated in Table 6.2; BHB: Beta–hydroxybutyrate. Row means bearing different superscripts within a fixed factor significantly differ ($P < 0.05$)

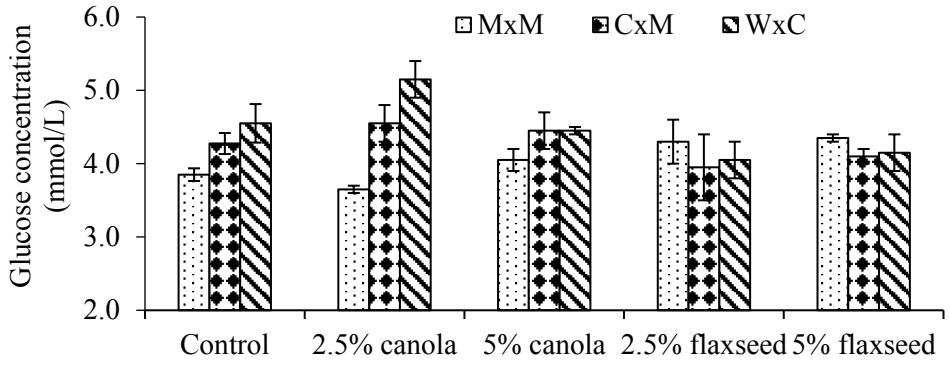

Figure 6.2. Variations in plasma glucose concentration as influenced by oil supplementation and breed interaction (MxM: Merino x Merino; CxM: Corriedale x Merino; WxC: White Suffolk x Corriedale)

6.5. Discussion

In Australia and New Zealand, food can be classified as a 'source' of n-3 PUFA if it contains at least 30 mg of EPA + DHA per standard serve and can be a 'good source' if it contains no less than 60 mg of EPA + DHA per standard serve (FSANZ, 2012) . A standard serve of red meat for Australian adults is reported to be 135 g (Hopkins et al., 2014; Ponnampalam et al., 2014a). Our study has demonstrated that hearts from lambs fed oil supplemented pellets could be a source of n-3 PUFA, although oil supplementation did not significantly influence EPA + DHA content. Furthermore, lamb kidneys and livers also could be labelled as good sources of n-3 PUFA. Our research is in agreement with other studies that have assessed FA profile of edible by-products from lamb (Malau-Aduli et al., 2016) and goat (Umaraw et al., 2015) and have reported that the total n-3 LC-PUFA content of liver and kidney were greater than that of heart.

The main traditional source of n-3 LC-PUFA is seafood including fish, crustaceans and molluscs, however, different types of seafood contain widely different amounts of n-3 LC-

PUFA (Nichols et al., 2010). In a typical seafood serve size (150 g raw), white-fleshed Australian wild-caught fish contain approximately 350 mg n-3 LC-PUFA (233 mg/100 g raw), while shellfish and prawns have about 225 (150 mg/100 g raw) and 180 mg (120 mg/100 g raw) respectively (Nichols et al., 2010). n-3 LC-PUFA content of liver in the present study was generally greater than that of white-fleshed Australian wild fish. Furthermore, the contents of n-3 LC-PUFA in lamb kidney and prawns were similar. Thus, liver and kidney may be considered as alternative and 'good sources' of n-3 LC-PUFA.

Several epidemiological studies have demonstrated that consumption of the less studied n-3 LC-PUFA - DPA - is positively correlated with lower incidence of coronary heart diseases and platelet aggregation (Mozaffarian et al., 2013; Byelashov et al., 2015), improvement in lipid metabolism, and inhibition of inflammation (Chen et al., 2012). Furthermore, Byelashov et al. (2015) stated that DPA improves cognitive function and mental health. Red meat contains relatively high levels of DPA compared to fish (Howe et al., 2007) and plays a major part in many diets (Rahmawaty et al., 2013; Ponnampalam et al., 2016b). In the present study, it is evident that the content of DPA in heart, kidney and liver was higher than those of EPA and DHA. Howe et al. (2006) stated that DPA contributes approximately 30% of total n-3 LC-PUFA in Australian diets. Therefore, it is being increasingly discussed that DPA should be included in total n-3 LC-PUFA intake (Howe et al., 2006; Mozaffarian and Wu, 2012; Byelashov et al., 2015). In fact, the Australian and New Zealand governments have offered guidelines for DPA intake along with EPA and DHA (NHMRC, 2005). Including DPA in n-3 LC-PUFA intake would boost the total n-3 LC-PUFA content of lamb to higher values (Howe et al., 2007; Clayton, 2014) which is in agreement with our findings.

It is now widely accepted that lipid supplementation of ruminant diets is an effective approach to modify edible fats. Feeding canola oil or flaxseed oil is a straightforward way to increase n-3 LC-PUFA in ruminant meat (Bessa et al., 2015). Nguyen et al. (2017) also concluded that

supplementing 5% of these oils (via supplementation) in prime lamb diets increased n-3 LC-PUFA contents of *Longissimus* muscle. Indeed, the inclusion of 5% flaxseed oil in this study increased the relative composition and content of liver DHA and kidney DPA, and total n-3 PUFA concentration in both liver and kidney in comparison with the control diet. These increases could be due to the increase in the intake and the ruminal concentration of ALA which increased in diet (Adeyemi et al., 2016a; Nguyen et al., 2017). Dietary ALA is an important n-3 PUFA that is used as a precursor for the production of tissue n-3 LC-PUFA including EPA, DPA and DHA. Research has shown that ruminant n-3 LC-PUFA concentration can be improved by increased dietary ALA intake from ALA-abundant vegetable oils (Bessa et al., 2007; Jerónimo et al., 2010). Our findings are in agreement with Adeyemi et al. (2016a) who found significant increases in the EPA and total n-3 PUFA concentration of liver when supplementing up to 8% with a blend of 80% canola oil and 20% palm oil in goat diets. Similarly, Kashani et al. (2015) investigated the FA composition of different tissues in dual-purpose Australian lambs and concluded that liver EPA and total n-3 PUFA concentration were significantly influenced by *Spirulina* supplementation. Kim et al. (2007) and Demirel et al. (2004) also reported the n-3 LC-PUFA concentration of lamb liver increased when dietary ALA intake was increased. Therefore, our results, in combination with previous studies, indicate that tissue n-3 LC-PUFA concentration is influenced by the level of dietary ALA intake.

Ruminant FA profiles are also directly associated with the extensive biohydrogenation of dietary MUFA and PUFA conducted by ruminal microbes. In the rumen, ALA is hydrogenated to rumelenic acid (cis-9, trans-11, cis-15 18:3) and subsequent reduction via rumenic acid (cis-9, trans-11 18:2), then to vaccenic acid (trans-11 18:1) and finally to stearic acid (18:0) (Wilde and Dawson, 1966) with an extent of 85–100% (Doreau and Ferlay, 1994). Furthermore, Doreau and Ferlay (1994) stated that lipid digestibility in the small intestine is independent of

the level of FA intake. This could explain the absence of significant oil supplementation effects on the n-3 LC-PUFA contents of heart, liver and kidney in our study.

Our findings indicated that variations in the FA profiles of editable organs were influenced by lamb breed, a finding that can be summarised as the individual n-3 LC-PUFA concentration of heart and kidney differed between MxM and WxC lambs. These variations could be explained by differences occurring in fatty acid metabolism, lipogenesis and deposition arising from genetic variation, as indicated by previous studies (Malau-Aduli et al., 2000; Wachira et al., 2002). This interpretation is supported by Sanudo et al. (2000) who found Merino lambs had less EPA and DPA concentration than coarse wool breeds. In contrast, Hoffman et al. (2013) did not observe differences in the EPA and DPA concentration of cooked heart, liver and kidney between Merino and Dorper lambs. The possible explanation for the observed differences between our study and that of Hoffman et al. (2013) is the status of investigated tissues. Tissue FA profiles could be changed during cooking (Maranesi et al., 2005; Flakemore et al., 2017). In the present study, lamb breed did not affect total n-3 PUFA concentration and n-3 LC-PUFA contents, consistent with the report of Malau-Aduli et al. (2016). Various studies stated that the effects of variations in n-3 LC-PUFA due to lamb breed were much more complicated and of a lower magnitude than variations due to the nutritional background (Sinclair, 2007; Ponnampalam et al., 2014a; Malau-Aduli et al., 2016).

The results of our study found no significant effect of supplementing enriched omega–3 oils on plasma cholesterol concentration in contrast to Obeidat et al. (2012) and Bhatt et al. (2013) who concluded that increases in cholesterol occurred. The variations in forms and higher dosages of fat sources used in these previous studies could explain the observed differences. Various studies reported that lipid supplementation did not influence the glucose and urea concentrations of ruminant plasma (Mirzaei et al., 2009; Otto et al., 2014; Lv et al., 2016). In agreement with these studies, our results showed that the inclusion of canola oil or flaxseed oil did not significantly alter the plasma concentrations of glucose and urea. An explanation for

the absence of significant differences in plasma metabolite concentrations could be related to the similarity of metabolisable energy components between the diets used in our study. Second, the oil levels used in this study were at or below 50 g/kg dry matter intake (DMI) to avoid the possibility of negative effects on DMI and nutrient digestibility (Wachira et al., 2000).

In agreement with our results, Malau-Aduli and Holman (2015) and Malau-Aduli et al. (2014) found that differences in plasma glucose between lamb breeds were observed. In beef cattle, Stahlhut et al. (2006) also reported Angus cows had greater plasma glucose concentrations than Simmental cows and concluded that plasma glucose concentration was influenced by average daily gain, milk production and FA profile of carcass. Differences in average daily gain (Hopkins et al., 2007) and FA profile (Malau-Aduli et al., 2016; Flakemore et al., 2017) have been reported between purebred Merino and crossbred lambs, indicating a potential for differences in plasma glucose concentration between the breeds. The high concentration of plasma urea in the present study could be due to high crude protein levels in the diets. Butler (2000) and Sunny et al. (2007) stated that the high dietary intake of protein and the balance of protein fractions present in the rumen can result in increased blood concentrations of urea.

Nguyen et al. (2017) reported the nutritional value, lipid percentage, fatty acid profile and sensory characteristics of visceral adipose and muscle tissues in these same 60 lambs used in the present study. They demonstrated that a standard serve (135 g) of meat produced from lambs supplemented with 5% oil contained more than 30 mg of EPA + DHA, reached the claimable 'source' level of n-3 LC-PUFA and significantly affected meat tenderness, juiciness and overall liking by human meat consumers. The finding in the current study that lamb liver and kidney also reached the claimable 'source' level of n-3 LC-PUFA has potential human health implications from the viewpoint of their EPA, DHA and DPA contents. In the n-3 synthesis pathway, DPA is an intermediary between EPA and DHA (Kaur et al., 2016). Many epidemiological studies in humans have demonstrated that DPA consumption is positively correlated with lower incidence of coronary heart diseases and lower platelet aggregation

(Phang et al., 2009; Mozaffarian et al., 2013), and in hamsters, inhibition of inflammation and improvement in lipid metabolism (Chen et al., 2012). Furthermore, Lim et al. (2013) stated that DPA improves mental health and spinal cord injury in mice, but the roles of DPA in human health have been largely ignored, perhaps because it is a negligible component of commercial fish and oil products compared to the n-3 LC-PUFA - EPA and DHA (NHMRC, 2006; Byelashov et al., 2015). Docosapentaenoic acid contributes approximately 30% of total n-3 LC-PUFA in human diets (Howe et al., 2006). It can serve as a reservoir for EPA and DHA because it is either retro-converted to EPA or elongated to DHA (Miller et al., 2013; Howes et al., 2015). Thus, some reports have suggested that DPA should be included in LC-PUFA intake (Howe et al., 2006; Mozaffarian and Wu, 2012). Indeed, Australia and New Zealand have offered guidelines for DPA intake along with EPA and DHA (NHMRC, 2006). Including DPA in n-3 LC-PUFA intake would boost the total n-3 LC-PUFA content produced in lamb meat to higher values (Clayton, 2014).

6.6. Conclusions

The results clearly demonstrated that both canola and flaxseed oils can be effectively used in feedlot regimes in the prime lamb industry without any detrimental health effects. For prime lamb producers wanting to achieve better product quality, in terms of FA composition, during the 10-week intensive finishing phase, supplementing 5% flaxseed oil into the animal diets considerably improves the content of the health benefitting n-3 LC-PUFA in the edible visceral organs. On the basis of the favourable FA content findings presented, lamb liver and kidney could be consumed as alternative ‘good sources’ of n-3 LC-PUFA.

Chapter 7: General Discussion and Conclusion

Most animal production traits and health status parameters are influenced by both genetic and environmental factors (De Smet et al., 2004). Berry et al. (2014) stated that genetics through crossbreeding strategies, is known to contribute to variation in animal performance, including growth rate and meat quality traits. Studies have reported breed differences for growth, carcass, meat eating and wool quality traits in the Australian sheep industry (Ponnampalam et al., 2009; Flakemore et al., 2015; Walkom and Brown, 2017). The environmental factors mainly include nutrition and management, but feeding strategies were employed in the majority of studies attempting to achieve greater animal performance (Meale et al., 2015; Burnett et al., 2017) and meat quality parameters (FA profiles sensory values) (Francisco et al., 2015; Ponnampalam et al., 2016b) that correspond better to current human nutrition recommendations.

The studies presented in this thesis tested the overarching hypothesis that different breeds, genders and the supplementation of n-3 PUFA oil sources enriched into wheat-based pellets would independently affect, and have interaction effects on Australian dual-purpose prime lamb productivity and product quality. To best investigate this hypothesis, several initial objectives were posed – in the **Introduction** – which gave rise to subordinate hypotheses that contribute to overall comprehension. These studies evaluated the effects of breed, gender, supplementing with graded levels of canola and flaxseed oils in prime lamb diets, and their interactions on:

1) Feed intake, growth rate and body conformation;
2) Wool quality;
3) Carcass traits;
4) Fatty acid profiles of muscle, adipose, heart, kidney and liver tissues and organs;
5) Meat eating quality;

6) Plasma metabolites.

The daily feed intake, growth rate and body conformation responses of Australian dual-purpose ewe and wether prime lambs from three different breeds to graded levels of either canola oil or flaxseed oil supplementation were assessed in **Chapters 3** and **4**. It was found that the inclusion of up to 5% of either canola oil or flaxseed oil in dual-purpose prime lamb diets did not affect dry matter feed intake and pre-slaughter productivity. The absence of significant variation in FE, ADG, LWT and body conformation could be attributed to level of oil supplementation and the similarity in ME (isocaloric) and CP (isonitrogenous) between experimental diets. Various studies have demonstrated that including oil up to 6% DM in isoenergetic and isonitrogenous lamb diets did not result in any negative effects on DMI and growth traits (Malau-Aduli et al., 2014; Meale et al., 2015; Dávila-Ramírez et al., 2017). Gender influenced WH, with wethers having greater WH than ewes at the same liveweight. Several studies had previously found that there was a significant impact of gender on WH (Holman et al., 2012, 2014a). This finding could be explained by the difference in body sizes between genders which is linked to hormonal variations between the sexes.

Lamb breed resulted in significant differences in feed intake, CG and BCS, with WxC lambs having markedly greater DMI, CG and BCS than CxM and purebred Merino lambs. These findings were consistent with those reported in some previous studies (Ríos et al., 2011; Malau-Aduli et al., 2012b; Holman et al., 2014a). Differences in gastro-intestinal tract capacity (Dillon et al., 2003, Boujenane, 2015), variations in genetic disposition towards muscle growth, wool growth or body fat deposition (Mitchell, 2007; Rodríguez et al., 2011) and the diversity in production type between breeds (Ekiz et al., 2009) could explain these findings. Malau-Aduli et al. (2012a) discussed that the partitioning of absorbed dietary nutrients towards synthetic pathways that favour muscle development or wool growth varies between breeds of sheep; thus purebred Merinos have a favourable disposition towards very fine wool production as opposed

to crossbreds that produce more meat at the expense of wool. There were significant effects of oil supplementation and its interaction with lamb breed on FE, ADG, LWT and CG, with crossbred lambs fed pellets containing 5% oil recording the greatest performance. These findings agree with other research investigations by Van Beem et al. (2008) and Holman et al. (2014a). Hegarty et al. (2006a) stated that the interaction effects of nutrition and genetics provide lamb producers with a wider range of choices of nutritional regimen and breed combinations to meet a vast array of market demands.

It was pertinent to investigate if supplementation of dual-purpose lambs with n3-PUFA oil is correlated with a downgrading of wool quality outcomes since nutrient partitioning seemed to favour average daily gain, liveweight, chest girth and muscle growth. Therefore, several indicative wool quality parameters were evaluated in **Chapter 3**. Wool production and quality are mainly influenced by the quality and amount of sulphur proteins such as cysteine and methionine (Khan et al., 2012). The addition of canola oil or flaxseed oil did not alter the CP content in lamb diets. As a result, wool quality traits were not influenced by n-3 PUFA oil supplementation in this study. Similar findings were observed by Malau-Aduli et al. (2014) who supplemented up to 5% canola oil in lamb finishing diets. There were no gender differences as similar wool quality traits were observed between males and females with the exception of FSD. In contrast, lamb breed proved to have a significant effect on wool quality with purebred Merino lambs producing the finest wool in this study. Possible explanations for the wool quality differences include variations in the trends of genetic disposition towards wool synthesis or muscle growth (Mitchell, 2007; Rodríguez et al., 2011), and wool follicle density between breeds (Adams and Cronjé, 2003). There were no significant second-order interactions on wool quality traits. Phenotypic correlations between wool quality traits were significant and ranged from moderate to very strong. The fact that the relationships between wool quality traits and lamb productivity measurements were non-significant implies that lamb wool and growth

performance traits are inherited independently, hence they can both be selected in breeding programmes without any fear of wiping off the gains in one by the other. On the other hand, if there were significant negative correlations between wool quality and growth performance, any gains made by selecting for high lamb productivity would be detrimental to wool quality.

Having demonstrated that there were no detrimental impacts of oil supplementation on wool quality while favourably promoting FE, LWT, ADG, and CG in the preceding Chapters, the research question that remained to be answered was: "What is the impact of n-3 PUFA oil supplementation and breed on carcass traits?" Therefore, **Chapter 4** investigated and provided an answer. It was found that the inclusion of graded levels of dietary canola oil or flaxseed oil did not influence dual-purpose prime lamb carcass parameters. These findings were consistent with previous studies (Radunz et al., 2009; Ferreira et al., 2014; Meale et al., 2015) supplementing fat up to 6% DM into lamb diets. Between lamb breeds, crossbreds had greater HCW, CCW and DP than the purebred Merinos. Interaction effects between oil supplementation and lamb breed on carcass traits were not significant. Nevertheless, it was able to answer the previous question that oil supplementation had no destructive effects on lamb carcass characteristics. Conversely, lamb breed was an independent source of variation in carcass traits, with crossbred lambs producing more meat.

Fatty acid analyses can provide insight into both associated sensory and nutritional qualities (Wood et al., 2008). The edible tissue FA profiles affected by oil inclusion and lamb breed were examined in **Chapters 5** and **6**. Five edible tissue and organ types were assessed including LTL muscle, visceral adipose, heart, kidney and liver.

The n-6/n-3 ratios of the measured tissues in this study were less than 5/1, which is a desirable dietary recommendation for human consumption (WHO/FAO, 1994). The inclusion of 5% flaxseed oil in lamb diets significantly reduced the n-6/n-3 ratios of all the measured tissues. Furthermore, lambs fed pellets containing 5% flaxseed oil significantly increased the ALA,

DPA, total PUFA proportions and PUFA/SFA ratio of the visceral adipose tissue. The total n-3 PUFA proportion of the visceral adipose, liver and kidney tissues were increased when lambs were offered 5% flaxseed oil pellets in comparison with the control diet. This is due to the greater amount of ALA and lower 18:2n-6 content in the 5% flaxseed oil supplementary diet than in the other diets. Sinclair et al. (2005) stated that part of the dietary-derived n-3 PUFA escaped biohydrogenation in the rumen when animals were fed diets containing abundant n-3 PUFA levels. Cooper et al. (2004) also concluded that the FA contents of animal products reflect their dietary levels. As a consequence, the addition of 5% flaxseed oil to lamb diets would increase the total n-3 PUFA proportions of measured tissues and thereby contribute to a decrease in the n-6/n-3 ratio.

The EPA + DHA and total n-3 LC-PUFA contents of the muscle tissue improved when lambs were supplemented with pellets containing either 5% canola oil or 5% flaxseed oil compared with lambs fed the control pellets. Furthermore, the inclusion of 5% flaxseed oil increased the concentration and content of liver DHA, kidney DPA and total n-3 PUFA in both liver and kidney in comparison with the control diet. A possible explanation for the increased n-3 PUFA content and concentration is the further biosynthesis by elongation of dietary-derived ALA to n-3 LC-PUFA by ruminal microbes. Sinclair (2007) stated that up to 30% of n-3 LC-PUFA flow in the small intestine is microbial n-3 LC-PUFA. These FA are directly absorbed in the intestine and stored in the muscle.

According to FSANZ (2012), if a standard serve of 135 g red meat contains at least 30 mg of EPA + DHA, it is labelled as a ‘source’ of n-3. It is a ‘good source’ if it contains no less than 60 mg of EPA + DHA. The findings in this thesis indicated that the muscle tissue produced from lambs fed pellets containing 5% oil consistently reached the claimable ‘source’ level of n-3. The heart tissues of lambs fed oil supplemented pellets could also be classified as a ‘source’

of n-3. Moreover, lamb liver and kidney could be considered as alternative 'good sources' of n-3. Thus, the addition of 5% canola oil or flaxseed oil to lamb diets would boost the EPA + DHA contents of sheep meat and edible visceral organs to higher values.

DPA is considered as an intermediate in the n-3 synthesis pathway, and serves as a reservoir for both EPA and DHA (Howes et al., 2015; Kaur et al., 2016). Its roles in human health have been well demonstrated and include its positive correlations with lower platelet aggregation, lower incidence of CVD, improvement in mental health and lipid metabolism, and inhibition of inflammation (Chen et al., 2012; Mozaffarian et al., 2013; Byelashov et al., 2015). However, various health organisations do not presently include DPA in their recommendations of n-3 LC-PUFA intakes (Byelashov et al., 2015). It was evident from this study that the content of DPA in muscle and heart tissues was similar with that of EPA and greater than DHA content. Moreover, the contents of DPA were greater than those of EPA and DHA in visceral fat, kidney and liver. Thus, DPA was a considerable part of the total n-3 LC-PUFA contents of the measured tissues. If DPA was classified as a health-beneficial n-3 LC-PUFA and included in the recommendations of n-3 LC-PUFA intakes, the nutritional values of sheep meat and edible visceral organs would be improved (Clayton, 2014).

It was demonstrated herein that lamb breed was a major source of variation in the FA profile of measured tissues. Crossbred lambs had greater EPA and DHA contents in the muscle than purebred Merino lambs. Moreover, there were significant differences in individual n-3 LC-PUFA concentration and contents of heart and kidney between purebred Merinos and crossbred lambs. In agreement with these results, Sanudo et al. (2000) found that Merino lambs had lower EPA and DPA concentration than coarse wool breeds. A number of studies also observed significant impacts of bovine breeds on tissue FA composition (Malau-Aduli et al., 1998; Malau-Aduli et al., 2000). Variations in fatty acid metabolism, lipogenesis and deposition between breeds could explain the genetic impacts on FA profiles (Malau-Aduli et al., 2000;

Wachira et al., 2002). However, the effect of lamb breed on n-3 LC-PUFA has a lower magnitude and is much more complicated than nutritional effect (Sinclair, 2007; Ponnampalam et al., 2014a; Malau-Aduli et al., 2016). Therefore, future emphasis on feeds and feeding strategies is required for further understanding of the key nutritional aspects and underlying factors behind the improvement of n-3 LC-PUFA profiles of edible tissues.

Sensory quality attributes play a critical role in the acceptability of commercial foodstuffs (Morales and Tsimidou, 2000). The effects of oil supplementation and lamb breed on meat eating quality characteristics were evaluated in **Chapter 5**. The results found that the inclusion of up to 5% oils rich in PUFA had no effect on meat sensory quality. Similar findings were also observed by Jerónimo et al. (2012) and Dávila-Ramírez et al. (2017). Tenderness, juiciness and overall liking of meat were influenced by lamb breed. Pannier et al. (2014a) and Hopkins et al. (2005) found that lamb breed had significant variation in meat sensory characteristics. The differences in muscle FA profile, IMF content, growth performance and maturity, which are influenced by genetics, could partly explain the effects of lamb breed on sensory quality traits (Hopkins, 2016).

Plasma metabolites are important indicators of individual health, nutritional status and animal productivity (Braun et al., 2010; Kholif et al., 2016). Thus, evaluating the variations in plasma metabolites in lambs as influenced by nutrition and genetics is critical (Hegarty et al., 2006b). In **Chapter 6,** the effects of oil supplementation and lamb breed on plasma metabolite concentrations was examined. The effect of dietary fat supplements on plasma metabolites has been associated with their ability to provide adequate metabolite precursors in the rumen (Howlett et al., 2003; Lake et al., 2006) and capacity for adaptation of animals to new diets (Schwaiger et al., 2013). In this study, no significant effect of oil supplementation on plasma metabolites was observed. The lack of significance in the present result indicates that the dietary oil treatment supplied adequate metabolite precursors to the rumen to maintain normal

plasma metabolite concentrations. Additionally, if the supplemented oil levels were at or below 5% DM, the effect of biohydrogenation of supplementary oil in the rumen was not any different between diets (Bhatt et al., 2016). Hence, the availability and levels of substrates necessary for normal metabolism of plasma metabolites were similar in all lambs.

In agreement with our results, Malau-Aduli and Holman (2015) and Malau-Aduli et al. (2014) found that different lamb breeds resulted in a significant variation in plasma glucose. Stahlhut et al. (2006) reported that plasma glucose concentration was influenced by ADG, milk production and FA profile of carcass. Differences in ADG and FA profiles between purebred Merino and crossbred lambs in this study indicate a potential for differences in plasma glucose concentration between the breeds.

Together, these findings demonstrated that both canola oil and flaxseed oil can be effectively used in dual-purpose prime lamb production systems during a 10-week feedlot period without any detrimental effect on animal performance, wool quality, carcass characteristics, sensory attributes of meat eating quality and plasma metabolites. Furthermore, supplementing 5% flaxseed oil or canola oil into animal diets considerably depressed the n-6/n-3 ratio and improved the content of health benefitting n-3 LC-PUFA in their meat and edible visceral organs. On the basis of the favourable FA content findings presented, meat produced from lambs supplemented with 5% oil in this study contained more than 30 mg of EPA+DHA and reached the claimable 'source' level of n-3 LC-PUFA. Lamb liver and kidney could be consumed as alternative 'good sources' of n-3 LC-PUFA.

Independent to oil supplementation, it was shown that sire breed and gender are imperative to influencing lamb productivity and product quality. This statement is based on the variations in lamb growth, carcass, wool and meat eating quality, FA profiles of edible tissues and plasma metabolites observed in response to these effects. Significant interactions between oil

supplementation and lamb breed on ADG and feed conversion ratio, visceral adipose and heart FA composition and meat juiciness score were detected.

The findings of this thesis will aid Australian dual-purpose prime lamb producers, research scientists and lamb consumers when:

- Selecting alternative n-3 PUFA-rich feed supplements which do not have negative impacts on animal health, productivity and sensory attributes of meat eating quality.
- Supplementing n-3 PUFA-rich sources at a cost-effective level to capitalise on optimal lamb liveweights, growth and the content of health-benefit n-3 LC-PUFA in meat and other edible tissues during intensive finishing periods.
- Reaffirming to the producers that oil supplementation does not negatively affect lamb health or productivity, as indicated by plasma metabolite concentrations.
- Manipulating flock genetics (breeds) to achieve desired productivity and product quality goals.
- Allowing the producers to match and manage lamb breeds with available feed resources to maximise profitability and meet targets specified by market demands.
- Reassuring the scientists that DPA accounts for a considerable part of total n-3 LC-PUFA contents in LTL muscle and heart, visceral fat, kidney and liver. Thus, DPA should be included in LC-PUFA intake.
- Demonstrating to consumers that lamb liver and kidney should be consumed as alternative ‘good sources’ of n-3 LC-PUFA.

It is necessary to perform further studies that focus on:

(1) the characterisation of the desaturations and elongations of the n-3 PUFA pathway to enable the manipulation of enzymes that enhance the content of n-3 LC-PUFA in ruminant products;

(2) comprehensively determining the pathways for the BH of n-3 LC-PUFA in the rumen;

(3) investigating protection technologies against the BH of dietary UFA to attain further enhancement of lamb meat n-3 PUFA;

(4) more deeply understanding the biological role and necessity of DPA in both animal nutrition and human health; and

(5) supplementing health-beneficial vegetable oils and their blends with other unsaturated oil sources (marine oils) to other prime and dairy lamb breed at under different management conditions.

In conclusion, it was evident that canola and flaxseed oil supplementation, both independently and in interaction with breed, influenced productive performances and product quality in Australian dual-purpose prime lambs. Similarly, breed and gender were shown to affect these same qualities independent to supplementation with oil. Therefore, the overarching hypothesis that either canola oil or flaxseed oil supplementation will alter purebred Merino and crossbred lambs' productive performances, and enhance health and product quality to varying extents dependent on breed and gender, can be accepted.

Limitations of the study:

- Feed costs associated with each supplementary diet were not computed. Therefore, it is difficult to compute profitability margins. Future research to determine the viability and profitability of sheep meat production with desired content of n-3 LC-PUFA using oil based supplements will fill this knowledge gap.
- The three sheep breeds utilised in this study represented a variety of common genotypes in the Tasmanian and Australian sheep industry. However, not all strains within the same breed (such as Merino) and other sheep breeds were investigated, thus limiting extrapolation of findings and the need to interpret with caution.

- Climatic conditions and production systems practiced in Northern Tasmania may not be generally applicable to all Australian States. Therefore, similar research in grazing systems obtainable in other parts of the country will provide insights and validation of the findings reported herein.

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Appendices

This section contains all the information on published chapters, supplementary data and declarations.

Appendix 1

Growth response of purebred Merino and crossbred prime lambs supplemented with canola and flaxseed oils

Nguyen V. D.[*1,2,] *S. W. Ives*[1], *R. W. Smith*[1] *& A. E.O. Malau-Aduli*[1,3]
[1]*University of Tasmania, Private Bag 98, Hobart, TAS 7001, Australia,* [2]*National Institute of Animal Science, Ha Noi, Viet Nam,* [3]*James Cook University, Townsville, QLD 4781, Australia.*

Abstract

The objective of this study was to evaluate the influence of flaxseed and canola oil supplementation on the growth of genetically divergent prime lambs. Seventy-two weaned lambs from Corriedale sires mated to Merino dams (CxM), White Suffolk sires mated to Corriedale dams (WxC) and purebred Merino (MxM) were randomly distributed into six treatment groups. Each group were daily supplemented with 1 kg pellet per lamb either flaxseed or canola oil at no oil (control), 25ml/kg (low) and 50ml/kg (high) oil levels. Lambs had ad libitum access to lucerne hay and water over a ten-week period. Results demonstrated that while gender had an inconsequential impact, significant differences in average daily gain (ADG) were attributable to oil supplementation. Furthermore, the appropriate level of these oils should be 50ml/kg over a ten-week supplementation. Lamb breed exerted the most significant impacts on liveweight and body condition score with WxC lambs elicited the best growth performance in this study.

Keywords: flaxseed oil, canola oil, supplementation, prime lambs, growth.

**Corresponding author: V.D.Nguyen@utas.edu.au*

Appendix 2

Nutritional value and sensory characteristics of meat eating quality of Australian prime lambs supplemented with pelleted canola and flaxseed oils: Fatty acid profiles of muscle and adipose tissues

Don Viet Nguyen[1,2], Aaron Ross Flakemore[1], John Roger Otto[1], Stephen William Ives[3], Rowan William Smith[3], Peter David Nichols[4] and Aduli Enoch Othniel Malau-Aduli[1,5]

Authors details:
[1]Animal Science and Genetics,
School of Land and Food,
University of Tasmania,
Hobart, TAS 7001, Australia
[2]National Institute of Animal Science, Hanoi,
Vietnam

[3] Extensive Agriculture Centre,
Tasmanian Institute of Agriculture,
University of Tasmania Private Bag 1375,
Launceston,
TAS 7250, Australia

[4]CSIRO Food Nutrition & Bio-based Products,
Oceans & Atmosphere, PO Box 1538, Hobart,
TAS 7001, Australia

[5]Veterinary Sciences,
College of Public Health,
Medical and Veterinary Sciences, Division of
Tropical Health and Medicine, James Cook
University,
Townsville, QLD 4781, Australia

Correspondence:
A. E. O. Malau-Aduli, PhD
Animal Science and Genetics,
School of Land and Food,
University of Tasmania,
Sandy Bay, Private Bag 54,
Hobart, TAS 7001, Australia and
Animal Genetics and Nutrition, Veterinary
Sciences Discipline,
College of Public Health,
Medical and Veterinary Sciences, Division of
Tropical Health and Medicine, James Cook
University, Townsville, Queensland 4811.
Australia. Tel: ++61-7-4781-5339;
Fax: +61-7-4725-4785; +61-3-6226-7444;
E-mail:aduli.malauaduli@jcu.edu.au

Abstract

The effects of canola or flaxseed oil dietary supplementation on *Longissimus thoracis et lumborum* (LTL) muscle and visceral adipose tissue fatty acid (FA) profiles and meat sensory traits in Australian prime lambs from different breeds were investigated. Sixty lambs were fed one of the following pellet treatments: no oil (Control), 2.5% canola, 5% canola, 2.5% flaxseed and 5% flaxseed, balanced by breed (purebred Merino, and first-cross lambs from Corriedale rams mated to Merino ewes and White Suffolk rams mated to Corriedale ewes). Lambs were individually supplemented daily with 1 kg of oil-enriched wheat-based pellets throughout the 7-week feeding trial, after a 3-week adjustment period and an unlimited access to water and lucerne hay. At the end of the feeding trial, all animals were slaughtered. From each carcass, an LTL muscle sampled at the 12/13th rib interface and a visceral adipose tissue sampled from the vicinity of the liver were taken and subjected to fatty acid analysis. A separate LTL muscle sample was utilised for sensory evaluation of meat eating quality. The inclusion of 5% flaxseed oil significantly decreased n-6/n-3 ratio in both tissues. The muscle from lambs fed 5% oil supplements had higher omega-3 long-chain polyunsaturated FA (n-3 LC-PUFA) contents and reached the claimable health-benefitting value without deleterious sensory effects. The n-3 LC-PUFA component in visceral adipose tissue was negligible. Tissue FA profiles and sensory quality were influenced by lamb breed. There were significant interactions between oil supplementation levels and lamb breed on some visceral adipose FA and meat juiciness. These findings indicate that a combination of dietary manipulation and lamb genetics can be used as an effective management tool to deliver a nutritionally improved n-3 LC-PUFA lamb to consumers.

Keywords: Omega-3 fatty acids; prime lamb; oil supplementation; muscle; adipose tissue; meat sensory eating quality

Appendix 3

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Growth performance and carcass characteristics of Australian prime lambs supplemented with pellets containing canola oil or flaxseed oil

Don V. Nguyen[A,B], *Bunmi S. Malau-Aduli*[C], *Peter D. Nichols*[A,D] *and Aduli E. O. Malau-Aduli*[A,E]

[A]Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, Qld 4811, Australia.
[B]National Institute of Animal Science, Hanoi, Vietnam.
[C]College of Medicine and Dentistry, Division of Tropical Health and Medicine, James Cook University, Townsville, Qld 4811, Australia.
[D]CSIRO Oceans & Atmosphere, PO Box 1538, Hobart, Tas. 7001, Australia.
[E]Corresponding author. Email: aduli.malauaduli@jcu.edu.au

Abstract. The objective of this study was to investigate the effects of enriched omega-3 oil supplemental pellets, breed and gender on lamb liveweight (LWT), body conformation and carcass characteristics, and to assess the relationships between body conformation and growth under an intensive finishing condition. Sixty ewe and wether prime lambs 7 months old were randomly allocated to one of five dietary treatments: no oil inclusion (Control); 2.5% canola oil; 5% canola oil; 2.5% flaxseed oil and 5% flaxseed oil, balanced by breed (purebred Merinos (M × M) and Corriedale × Merino (C × M) and White Suffolk × Corriedale (W × C) first crosses). Lambs were individually supplemented with 1 kg pellets per day and had free access to lucerne hay and water throughout the 7-week feeding trial, after a 3-week adaptation. Dietary oil inclusion did not cause significant differences in daily feed intake, growth performance and carcass characteristics ($P > 0.05$). However, first-cross W × C lambs had significantly higher feed intake, chest girth and body conformation score ($P < 0.05$) than M × M and C × M lambs. Carcass weight, dressing percentage and fat depth of crossbred lambs were significantly higher than those of M × M ($P < 0.05$). Significant interactions between oil inclusion and breed on average daily gain (ADG) and feed conversion ratio were observed. There were positive and highly significant correlations among LWT, ADG and body conformation measurements ($P < 0.01$). These findings suggest that prime lamb producers can better manage and match their breeding goals with feed resources by supplementing first-cross C × M lambs with pellets containing 5% canola oil or feeding first-cross W × C lambs with 5% flaxseed oil pellets during the 10-week intensive finishing period.

Additional keywords: body conformation, dressing percentage, feed intake.

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Appendix 4

nutrients

MDPI

Article

Omega–3 Long-Chain Fatty Acids in the Heart, Kidney, Liver and Plasma Metabolite Profiles of Australian Prime Lambs Supplemented with Pelleted Canola and Flaxseed Oils

Don V. Nguyen [1,2], Van H. Le [1,2], Quang V. Nguyen [1,3], Bunmi S. Malau-Aduli [4], Peter D. Nichols [1,5] and Aduli E. O. Malau-Aduli [1,*]

[1] Animal Genetics and Nutrition, Veterinary Sciences Discipline, College of Public Health, Medical and Veterinary Sciences, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; donvietnguyen@jcu.edu.au (D.V.N.); Vanhung@jcu.edu.au (V.H.L.); Quangvunguyen@jcu.edu.au (Q.V.N.); peterd.nichols@csiro.au (P.D.N.)
[2] National Institute of Animal Science, Thuy Phuong, Bac Tu Liem, Hanoi 129909, Vietnam
[3] College of Economics and Techniques, Thai Nguyen University, Thai Nguyen 252166, Vietnam
[4] College of Medicine and Dentistry, Division of Tropical Health and Medicine, James Cook University, Townsville, QLD 4811, Australia; bunmi.malauaduli@jcu.edu.au
[5] CSIRO Oceans & Atmosphere, P.O. Box 1538, Hobart, TAS 7001, Australia
* Correspondence: aduli.malauaduli@jcu.edu.au; Tel.: +61-7-4781-5339; Fax: +61-7-4779-1526

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Abstract: The objective of the study was to ascertain whether human health beneficial omega–3 long-chain (≥C_{20}) polyunsaturated fatty acid (*n*-3 LC-PUFA) content in heart, kidney and liver can be enhanced by supplementing prime lambs with graded levels of canola and flaxseed oil. Health status of the lambs, as a consequence of the supplementation, was also investigated by examining their plasma metabolites. Sixty purebred and first-cross lambs were allocated to one of five treatments of lucerne hay basal diet supplemented with isocaloric and isonitrogenous wheat-based pellets without oil inclusion (Control) or graded levels of canola oil at 2.5% (2.5C), 5% (5C), flaxseed oil at 2.5% (2.5F) and 5% (5F) in a completely randomised design. Pre-slaughter blood, post-slaughter kidney, liver and heart samples were analysed for plasma metabolite and fatty acid profiles. Summations of docosapentaenoic acid and docosahexaenoic acid, and total *n*-3 LC-PUFA were enhanced in the liver and kidney of 5F supplemented lambs with a marked decrease in *n*-6/*n*-3 ratio and significant breed differences detected. There were generally no deleterious impacts on animal health status. A combination of 5% oil supplementation and lamb genetics is an effective and strategic management tool for enhancing *n*-3 LC-PUFA contents of heart, kidney and liver without compromising lamb health.

Key words: prime lamb; oil supplementation; visceral organs; *n*-3 LC-PUFA; plasma metabolites

1. Introduction

The Australian Guide to Healthy Eating and Australian Dietary Guidelines [1] promote health and wellbeing by providing scientific evidence based dietary advice to reduce the risk of high cholesterol, high blood pressure, obesity, type 2 diabetes, cardiovascular disease and cancers. Therefore, consumers have become more aware and concerned about the relationship between dietary intake and health as their health consciousness increases. High levels of saturated fatty acids (SFA) and low content of polyunsaturated fatty acids (PUFA) in red meat have been implicated in the increased incidence of chronic diseases, especially cardiovascular diseases, diabetes and cancers [2,3]. Enser et al. [4] reported that lamb contains a higher fat percentage than beef and pork, and lower